(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 956 865 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
17.11.1999 Bulletin 1999/46

(21) Application number: **97934756.4**

(22) Date of filing: **08.08.1997**

(51) Int. Cl.[6]: **A61K 45/00**, A61K 31/16, A61K 31/165, A61K 31/195, A61K 49/00, A61K 31/445, A61K 31/50, A61K 31/495, A61K 31/44, C07D 213/81, C07D 401/12

(86) International application number:
PCT/JP97/02793

(87) International publication number:
WO 98/06433 (19.02.1998 Gazette 1998/07)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV RO SI

(30) Priority: **12.08.1996 JP 21240996**

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(54) MEDICINES COMPRISING Rho KINASE INHIBITOR

(57) A Rho kinase inhibitor is provided as a novel pharmaceutical agent, particularly as a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a prophylactic agent of immature birth, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a contraceptive, a prophylactic agent of digestive tract infection, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy and a brain function improving drug. In addition, the Rho kinase inhibitor is provided as a reagent and a diagnostic.

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Description

Technical Field

[0001] The present invention relates to treatment of various diseases by the use of a Rho kinase inhibitor as a pharmaceutical agent. Moreover, the present invention relates to use of a Rho kinase inhibitor as a reagent or a diagnostic.

Background Art

[0002] Ever since the discovery of Ras in 1981, a number of small GTP binding proteins (small G proteins) similar to Ras have been found, and many physiological functions they possess have been studied. These small G proteins have a molecular weight of 20,000-30,000 and do not have a subunit structure. They all specifically bind GDP and GTP, and hydrolyze the thus-bound GTP (GTPase activity) (Hall, A., Science, 249, 635-640, 1990; Bourne, H. R. et al., Nature, 349, 117-127, 1991).

[0003] To date, more than 50 kinds of genes encoding these small G proteins have been found from yeast to mammals, forming a superfamily. These small G proteins are largely divided into 5 groups of Ras, Rho, Rab, Arf and others, according to the similarity of amino acid sequences.

[0004] Of these, Rho was named so because its gene isolated in the form of cDNA from sea hare neuromuscle encodes a polypeptide having about 35% homology with Ras (Ras homologue) (Madaule, P., Cell, 41, 31-40, 1985).

[0005] Rho is specifically ADP ribosylated by C3 enzyme, which is one of the botulinum toxins, and Staphylococcal toxin EDIN, and inactivated (Narumiya, S. and Morii, S., Cell Signal, 5, 9-19, 1993; Sekine, A. et al., J. Biol. Chem., 264, 8602-8605, 1989). Hence, the C3 enzyme and EDIN were used to study the involvement of Rho in cell functions from various aspects.

[0006] For example, phosphorylation by myosin light chain (MLC) kinase is considered to enable actin · myosin interaction and initiate contraction of smooth muscle, and the structure of smooth muscle myosin phosphatase which dephosphorylates MLC has been clarified (Shimizu, H. et al., J. Biol. Chem., 269, 30407-30411, 1994). It has been clarified that the activity of myosin phosphatase is, like MLC kinase, under the control of the intracellular signal transduction system and Rho is involved in this mechanism. Moreover, an active Rho bound with GTP has been found to enhance Ca-dependent contraction in a smooth muscle skinned fiber specimen (Hirata, K., J. Biol. Chem., 267, 8719-8722, 1992), thereby suggesting that the increase in Ca sensitivity in smooth muscle contraction is caused by the inhibition of myosin phosphatase activity via Rho.

[0007] In Swiss 3T3 cell and 3Y1 cell, moreover, Rho-dependent promotion of tyrosine phosphorylation (Kumagai, N. et al., J. Biol. Chem., 270, 8466-8473, 1993) and activation of many kinds of serine/threonine kinases (Kumagai, N. et al., FEBS Lett., 366, 11-16, 1995) have been acknowledged. From this, the presence of plural protein kinases in the downstream of Rho in the signal transduction pathway via Rho has been suggested and, actually, ROCα (Leung, T. et al., J. Biol. Chem., 270, 29051-29054, 1995) [another name Rho-kinase, ROCK-II] and p160ROCK (Ishizaki, T. et al., The EMBO J., 15(8), 1885-1893, 1996) [another name ROCβ, ROCK-I] have been reported as serine/threonine kinase (Rho kinase) activated along with the activation of Rho. It has been also reported that biological distribution of the both enzymes is different (Nakagawa, O. et al., FEBS Lett. 392 189-193, 1996). In addition, it has been reported that this Rho kinase directly phosphorylates myosin phosphatase and inhibits its activity (Kimura, K. et al., Science, 273, 245-248, 1996).

[0008] Rho has been documented to be responsible for the activation of not only protein kinase but also lipid kinase (Zang, J. et al., J. Biol. Chem., 268, 22251-22254, 1993), and the presence of phospholipase (PLD) activated by Rho has been also suggested (Siddiqi, A. R. et al., J. Biol. Chem., 268, 24535-24538, 1995).

[0009] Control by Rho of the motility of Swiss 3T3 fibroblasts in the presence of serum, motility of keratinocyte 303R by HGF and TPA (12-O-tetradecanoylphorbol 13-acetate), spontaneously occurred and chemoatractant mediated motility of neutrophils have been reported (Takai, Y. et al., Trends Biochem. Sci., 20, 227-231, 1995), and control of the permeation of liver cancercell (MM1 cell), which is one of the metastatic cancer models, through cultured mesothelial layer by the activation of Rho has been reported (Yoshioka, K. et al., FEBS Lett., 372, 25-28, 1995), thereby suggesting the involvement of Rho in cell motility.

[0010] Meanwhile, in the cells derived from nerves, such as neuroblastoma, PC-12 cells and the like, retraction of neurite and rounding of the cell by lysophosphatidic acid, which is an activation stimulant of Rho, have been acknowledged. Inasmuch as this retraction can be inhibited by C3 enzyme treatment (Jalink, K. et al., J. Cell Biol., 126, 801-810, 1994) and the formation of ringed structure of podosome, which separates the site where dissolution and absorption of bone take place in the clear zone of osteoclast from the surrounding, is inhibited by C3 enzyme treatment (Zhang, D. et al., J. Cell Sci., 108, 2285-2292, 1995), a deep involvement of Rho in the morphological changes in cells has been suggested.

[0011] In addition, C3 enzyme treatment reportedly inhibits activation of an adhesion molecule such as LFA (leuko-

cyte function-associated antigen) and the like, and C3 enzyme treatment reportedly inhibits proliferation of Swiss 3T3 fibroblasts (Yamamoto, M. et al., Oncogene, 8, 1449-1455, 1993). Thus, Rho reportedly controls cell adhesion and cell division via actin cytoskeleton, and is also concerned with the transcription control of c-fos gene (Hill, C. S. et al., Cell, 81, 1159-1170, 1995) and transformation of cell (Khosravi-Far, R. et al., Mol. Cell Biol., 15(11), 6443-6453, 1995).

[0012] In view of the inhibition of invasion of dysentery bacillus into epithelial cells by C3 enzyme, a recent report has documented the deep involvement of Rho in bacterial infection (Adam, T. et al., The EMBO J., 15(13), 3315, 1996).

[0013] In pregnant rats, moreover, the levels of Rho and Rho kinase are reported to be higher as compared to non-pregnant rats (Niiro, N. et al., Biochem. Biophys. Res. Commun., 230, 356-359, 1997), and deep involvement of Rho and Rho kinase in muscle contraction of uterus for childbirth has been known. Further, integrin (Sueoka, K. et al., Fertility & Sterility, 67(5) 799-811, 1997) considered to be involved in the cell-cell and cell-extracellular matrix adhesion during the stages of fertilization, embryogenesis and embryonidation is known to be activated by Rho (Morii, N. et al., J. Biol. Chem., 267, 20921-20926, 1992).

[0014] Hence, it has been made clear that Rho is activated upon receipt of signals from various cell membrane receptors and the activated Rho functions as a molecule switch of a broad range of cell phenomena, such as smooth muscle contraction, cell motility, cell adhesion, morphological changes of cell, cell growth and the like, via actomyosin system.

[0015] Smooth muscle contraction is significantly involved in the disease states of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, imminent immature birth and the like; cell motility plays an important role in invasion and metastasis of cancer, arteriosclerosis, retinopathy, immune response and the like; cell adhesion is deeply involved in metastasis of cancer, inflammation, autoimmune disease, AIDS, fertilization and nidation of fertilized egg and the like; morphological change of cell is deeply involved in brain function disorder, osteoporosis, bacterial infection of digestive tract and the like; and cell growth is deeply involved in cancer, arteriosclerosis and the like. Therefore, a drug that blocks the functions of Rho is considered to make a therapeutic agent for these diseases in which Rho plays some role.

[0016] At present, however, only C3 enzyme and EDIN can inhibit the actions of Rho. These are proteins which cannot permeate cytoplasm, which prevents their development as a pharmaceutical agent.

[0017] On the other hand, inhibition of Rho kinase, which is considered to be present downstream of the signal transduction pathway via Rho, is considered to lead to the inhibition of responses of various cell phenomena due to Rho. However, a specific inhibitor of Rho kinase has not been known to date.

[0018] It is expected, therefore, that by searching a compound that inhibits Rho kinase, such Rho kinase inhibitor will be an effective agent for the prophylaxis and/or treatment of the above-mentioned diseases and phenomena relating to Rho, such as hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, immature birth, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, fertilization and nidation of fertilized egg, osteoporosis, retinopathy, brain function disorder, bacterial infection of digestive tract and the like.

[0019] The compound of the formula (I) is already known to be useful as an agent for the prophylaxis and treatment of circulatory disorder in coronary, cerebral, renal and peripheral arteries and the like (e.g., a potent and long lasting therapeutic agent of hypertension, angina pectoris, renal and peripheral circulation disorder, and suppressive agent of cerebrovascular contraction and the like), as well as a therapeutic agent of asthma (Japanese Patent Unexamined Publication No. 62-89679, Japanese Patent Unexamined Publication No. 3-218356, Japanese Patent Unexamined Publication No. 4-273821, Japanese Patent Unexamined Publication No. 5-194401, Japanese Patent Unexamined Publication No. 6-41080 and WO95/28387 and the like).

[0020] The compound of the formula (II) is already known to be useful as a vasodilator, a therapeutic agent of hypertension, a brain function improving agent, an anti-asthma agent, a heart protection agent, a platelet aggregation inhibitor, a psychosyndrome treating agent, an anti-inflammatory agent and an agent for the prophylaxis and treatment of hyperviscosity syndrome (Japanese Patent Unexamined Publication No. 57-200366, Japanese Patent Unexamined Publication No. 61-227581, Japanese Patent Unexamined Publication No. 2-256617, Japanese Patent Unexamined Publication No. 4-264030, Japanese Patent Unexamined Publication No. 6-56668, Japanese Patent Unexamined Publication No. 6-80569, Japanese Patent Unexamined Publication No. 6-293643, Japanese Patent Unexamined Publication No. 7-41424 and Japanese Patent Unexamined Publication No. 7-277979).

[0021] However, these compounds of the formula (I) or (II) are not known to block the functions of Rho or to have Rho kinase inhibitory action.

Disclosure of the Invention

[0022] The present invention aims at providing a Rho kinase inhibitor as a novel pharmaceutical agent. As a result of intensive studies, the present inventors have found that a compound inhibiting Rho kinase has an antihypertensive action, an anti-angina pectoris action, a cerebrovascular contraction suppressive action, an anti-asthma action, a peripheral circulation improving action, an immature birth preventive action, an anti-arteriosclerosis action, an anti-can-

cer action, an antiinflammatory action, an immunosuppressive action, an autoimmune disease improving action, an anti-AIDS action, a preventive action on fertilization and nidation of fertilized egg, an osteoporosis treating action, a retinopathy treating action, a brain function improving action, a preventive action on bacterial infection of digestive tract and that the Rho kinase inhibitor is useful as a pharmaceutical agent, particularly as a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a prophylactic agent of immature birth, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy, a brain function improving drug, a contraceptive and a prophylactic agent of digestive tract infection, which resulted in the completion of the present invention.

[0023] It has been also found that a compound which inhibits Rho kinase is useful as a reagent for the study of Rho and Rho kinase and as a diagnostic of the diseases relating to those, which resulted in the completion of the present invention.

[0024] Accordingly, the present invention provides the following.

(1) A pharmaceutical agent containing a Rho kinase inhibitor.

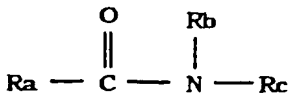
(2) A pharmaceutical agent containing a Rho kinase inhibitor, which is at least one member selected from the group consisting of a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy, a brain function improving drug, a prophylactic agent of immature birth, a contraceptive and a prophylactic agent of digestive tract infection.

(3) A pharmaceutical composition containing a therapeutically effective amount of a Rho kinase inhibitor and a pharmaceutically acceptable additive.

(4) A reagent containing a Rho kinase inhibitor.

(5) A diagnostic containing a Rho kinase inhibitor.

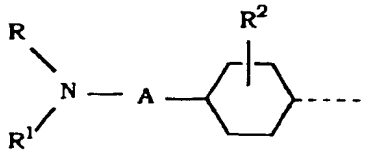
(6) A Rho kinase inhibitor containing an amide compound of the formula (I)



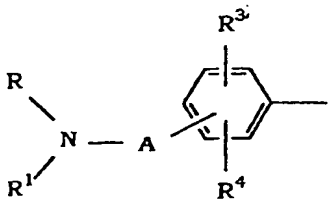
(I)

wherein

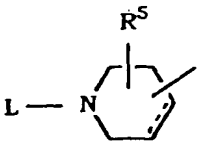
Ra is a group of the formula



(a)



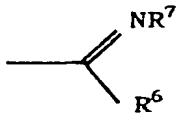
(b) or



(c)

in the formulas (a) and (b),

R is hydrogen, alkyl or cycloalkyl, cycloalkylalkyl, phenyl or aralkyl, which optionally have a substituent on the ring, or a group of the formula



(d)

wherein R^6 is hydrogen, alkyl or formula : $-NR^8NR^9$ wherein R^8 and R^9 are the same or different and each is hydrogen, alkyl, aralkyl or phenyl, R^7 is hydrogen, alkyl, aralkyl, phenyl, nitro or cyano, or R^6 and R^7 in combination show a group forming a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom,

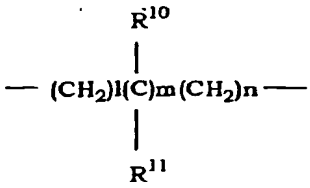
R^1 is hydrogen, alkyl or cycloalkyl, cycloalkylalkyl, phenyl or aralkyl, which optionally have a substituent on the ring, or

R and R^1 in combination form, together with the adjacent nitrogen atom, a group forming a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom,

R^2 is hydrogen or alkyl,

R^3 and R^4 are the same or different and each is hydrogen, alkyl, aralkyl, halogen, nitro, amino, alkylamino, acylamino, hydroxy, alkoxy, aralkyloxy, cyano, acyl, mercapto, alkylthio, aralkylthio, carboxy, alkoxycarbonyl, carbamoyl, alkylcarbamoyl or azide, and

A is a group of the formula



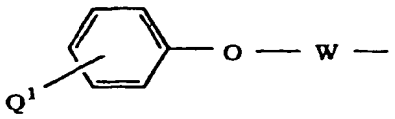
(e)

wherein R^{10} and R^{11} are the same or different and each is hydrogen, alkyl, haloalkyl, aralkyl, hydroxyalkyl, carboxy or alkoxycarbonyl, or R^{10} and R^{11} show a group which forms cycloalkyl in combination and l, m and n are each 0 or an integer of 1-3,

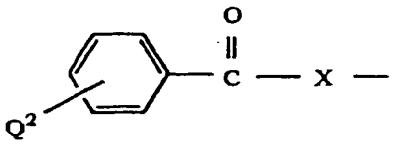
in the formula (c),

L is hydrogen, alkyl, aminoalkyl, mono or dialkylaminoalkyl, tetrahydrofurfuryl, carbamoylalkyl, phthalimidoalkyl, amidino or a group of the formula

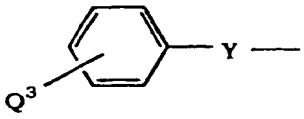
(f)



(g)



(h) or

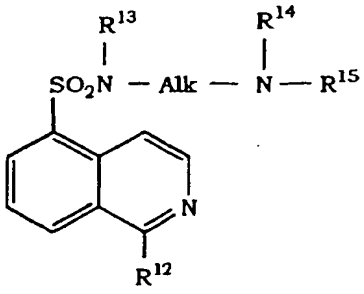


(i)

wherein B is hydrogen, alkyl, alkoxy, aralkyl, aralkyloxy, aminoalkyl, hydroxyalkyl, alkanoyloxyalkyl, alkoxycarbonylalkyl, α-aminobenzyl, furyl, pyridyl, phenyl, phenylamino, styryl or imidazopyridyl,
Q^1 is hydrogen, halogen, hydroxy, aralkyloxy or thienylmethyl,
W is alkylene,
Q^2 is hydrogen, halogen, hydroxy or aralkyloxy,
X is alkylene,
Q^3 is hydrogen, halogen, hydroxy, alkoxy, nitro, amino, 2,3-dihydrofuryl or 5-methyl-3-oxo-2,3,4,5-tetrahydropyridazin-6-yl; and Y is a single bond, alkylene or alkenylene, and
in the formula (c),
a broken line is a single bond or a double bond, and

R^5 is hydrogen, hydroxy, alkoxy, alkoxycarbonyloxy, alkanoyloxy or aralkyloxycarbonyloxy;
Rb is a hydrogen, an alkyl, an aralkyl, an aminoalkyl or a mono- or dialkylaminoalkyl; and
Rc is an optionally substituted heterocycle containing nitrogen, an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(7) A pharmaceutical agent containing a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof, which is a therapeutic agent of at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma and peripheral circulation disorder, which are caused by Rho kinase.
(8) A pharmaceutical agent containing a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof, which is at least one therapeutic agent selected from the group consisting of a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy, a brain function improving drug, a prophylactic agent of immature birth, a contraceptive and a prophylactic agent of digestive tract infection.
(9) A reagent having a Rho kinase inhibitory activity, which contains a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.
(10) A diagnostic of a disease caused by Rho kinase, which contains a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.
(11) A Rho kinase inhibitor containing a substituted isoquinolinesulfonamide derivative of the formula (II)



(II)

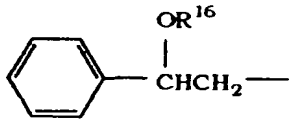
wherein

R^{12} is a hydrogen, a chlorine or a hydroxy, and
when R^{12} is a hydrogen,
Alk is an alkylene having 2 to 6 carbon atoms, which optionally has alkyl having 1 to 10 carbon atoms, aryl or aralkyl as a substituent;

R^{13} is a hydrogen;

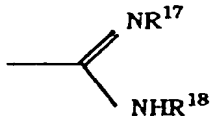
R^{14} is a hydrogen, or a linear or branched alkyl having 1 to 6 carbon atoms, an aryl or an aralkyl;

R^{15} is a hydrogen, a linear or branched alkyl having 1 to 6 carbon atoms, an aryl or an aralkyl, or a benzoyl, a cinnamyl, a cinnamoyl, a furoyl or a group of the following formula



(j)

wherein R^{16} is linear or branched alkyl having 1 to 6 carbon atoms or a group of the following formula



(k)

wherein R^{17} and R^{18} are hydrogen or directly bonded to form alkylene having 2 to 4 carbon atoms; or

R^{13} and R^{14} are directly bonded to form alkylene having 4 or less carbon atoms, which is optionally substituted by alkyl having 1 to 10 carbon atoms, phenyl or benzyl, or

R^{14} and R^{15} directly or in combination via oxygen atom form a heterocycle together with the adjacent nitrogen atom, and
when R^{12} is a chlorine or a hydroxy, Alk is an alkylene having 2 to 6 carbon atoms, which is optionally substituted at the hydrogen bonded to carbon by alkyl having 1 to 6 carbon atoms,

R^{13} and R^{14} are each a hydrogen, a linear or branched alkyl having 1 to 6 carbon atoms or directly bonded to each other to form ethylene or trimethylene, wherein hydrogen bonded to carbon is optionally substituted by alkyl having 1 to 6 carbon atoms; or

R^{15} is a hydrogen, a linear or branched alkyl having 1 to 6 carbon atoms or an amidino,
an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(12) A pharmaceutical agent containing a compound of the formula (II), an isomer thereof and/or a pharmaceuti-

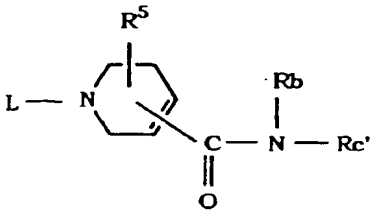
cally acceptable acid addition salt thereof, which is a therapeutic agent of at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, inflammation and, brain function disorder, which are caused by Rho kinase.

(13) A pharmaceutical agent containing a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof, which is at least one therapeutic agent selected from the group consisting of a therapeutic agent of peripheral circulation disorder, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy, a prophylactic agent of immature birth, a contraceptive and a prophylactic agent of digestive tract infection.

(14) A reagent having a Rho kinase inhibitory activity, which contains a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(15) A diagnostic for a disease caused by Rho kinase, which contains a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(16) A compound of the formula (III)



(III)

wherein Rc' is an optionally substituted heterocycle having nitrogen, which is other than pyridine of Rc, and other symbols are as defined above, an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(17) The pharmaceutical agent of the above (1), containing a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

(18) A pharmaceutical agent containing a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof, which is at least one therapeutic agent selected from the group consisting of a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy, a brain function improving drug, a prophylactic agent of immature birth, a contraceptive and a prophylactic agent of digestive tract infection.

(19) A pharmaceutical composition of the above (3), containing a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

(20) A reagent having a Rho kinase inhibitory activity, which contains a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

(21) A diagnostic for a disease caused by Rho kinase, which contains a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(22) A method for treating a disease based on inhibition of Rho kinase, comprising administering a pharmaceutically effective amount of a Rho kinase inhibitor to a patient.

(23) The treating method of the above (22), wherein the disease treatable by the inhibition of the Rho kinase is at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, a peripheral circulation disorder, arteriosclerosis, cancer, an inflammation, an immune disease, an autoimmune disease, AIDS, osteoporosis, retinopathy, a brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(24) A method for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma and a peripheral circulation disorder, which are caused by Rho kinase, and arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract, which comprises administering a pharmaceutically effective amount of a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(25) A method for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, inflammation and brain function disorder, which are caused by Rho kinase,

and a peripheral circulation disorder, arteriosclerosis, cancer, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract, which comprises administering a pharmaceutically effective amount of a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(26) A method for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain-function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract, which comprises administering a pharmaceutically effective amount of a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

(27) Use of a Rho kinase inhibitor for the production of a therapeutic agent of a disease treatable by inhibiting Rho kinase.

(28) The use of a Rho kinase inhibitor of the above (27), wherein the disease treatable by the inhibition of Rho kinase is at least one member selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(29) The use of a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof for the production of a therapeutic agent of at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma and peripheral circulation disorder caused by Rho kinase, and arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(30) Use of a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof for the production of a therapeutic agent of at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, inflammation and brain function disorder caused by Rho kinase, and peripheral circulation disorder, arteriosclerosis, cancer, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(31) Use of a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof for the production of a therapeutic agent of at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(32) A commercial package comprising a Rho kinase inhibitor and a written matter associated therewith, the written matter stating that the Rho kinase inhibitor can or should be used for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(33) A commercial package comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof and a written matter associated therewith, the written matter stating that the compound can or should be used for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma and peripheral circulation disorder, which are caused by Rho kinase, and arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(34) A commercial package comprising a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof and a written matter associated therewith, the written matter stating that the compound can or should be used for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma., inflammation and brain function disorder, which are caused by Rho kinase, and peripheral circulation disorder, arteriosclerosis, cancer, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

(35) A commercial package comprising a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof and a written matter associated therewith, the written matter stating that the compound can or should be used for treating at least one disease selected from the group consisting of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, arteriosclerosis, can-

cer, inflammation, immune disease, autoimmune disease, AIDS, osteoporosis, retinopathy, brain function disorder, immature birth, fertilization and nidation of fertilized egg and infection of digestive tract.

Detailed Description of the Invention

[0025] The Rho kinase inhibitory action, antihypertensive action, anti-angina pectoris action, cerebrovascular contraction suppressive action, anti-asthma action, peripheral circulation improving action, immature birth preventive action, anti-arteriosclerosis action, anti-cancer action, antiinflammatory action, immunosuppressive action, autoimmune disease improving action, anti-AIDS action, preventive action of fertilization and nidation of fertilized egg, preventive action on bacterial infection of digestive tract, osteoporosis treating action, retinopathy treating action and brain function improving action of the present invention can be confirmed by Rho kinase inhibitory activity, vasohypotonic action, trachea relaxing action, peripheral blood flow increasing action, cell adhesion induction inhibitory action, malignant tumor metastasis inhibitory action, bone resorption inhibitory action, mouse allogenic MLR inhibitory activity, tumor cell proliferation inhibitory action, angiogenesis inhibitory action, vascular smooth muscle cell proliferation inhibitory action and the like.

[0026] The disease relating to Rho, on which the inventive Rho kinase inhibitor is effective include, for example, disease symptoms of hypertension, angina pectoris, cerebrovascular contraction, asthma, peripheral circulation disorder, immature birth, arteriosclerosis, cancer, inflammation, immune disease, autoimmune disease, AIDS, bacterial infection of digestive tract, osteoporosis, retinopathy, brain function disorder and the like, as well as biological phenomena such as fertilization and nidation of fertilized egg.

[0027] As used herein, by the Rho kinase of the present invention is meant serine/threonine kinase activated along with the activation of Rho, which is exemplified by the aforementioned ROCα(ROCKII), p160ROCK(ROCβ, ROCK-I) and other proteins having serine/threonine kinase activity.

[0028] Cancer includes bone marrow leukemia, lymphocytic leukemia, gastric cancer, colon cancer, lung cancer, pancreatic cancer, liver cancer, cancer of esophangus, ovarian cancer, breast cancer, skin cancer, cervical cancer, orchioncus, neuroblastoma, urinary epithelial cancer, multiple myeloma, uterine cancer, melanoma, cerebral tumor and the like, and anti-cancer means inhibition of formation, infiltration, metastasis, growth and the like of these tumors.

[0029] The immune disease includes allergic diseases, rejection in organ transplantation and the like.

[0030] The autoimmune disease includes articular rheumatism, systemic lupus erythematodes, Sjögren's disease, multiple sclerosis, myasthenia gravis, type I diabetes, endocrine ophthalmopathy, primary biliary cirrhosis, Crohn's disease, glomerulonephritis, sarcoidosis, psoriasis, pemphigus, hyoplastic anemia, essential thrombocytopenic purpura and the like.

[0031] Bacterial infection of digestive tract means various diseases caused by the invasion of *Salmonella*, dysentery bacillus, intestinal pathogenic Escherichia coli and the like into intestinal mucosa epithelial cells.

[0032] Retinopathy means angiopathic retinopathy, arteriosclerosis retinopathy, central angiospastic retinopathy, central serous retinopathy, circinate retinopathy, diabetic retinopathy, dysproteinemic retinopathy, hypertensive retinopathy, leukemic retinopathy, lipemic retinopathy, proliferative retinopathy, renal retinopathy, sickle retinopathy, toxemic retinopathy of pregnancy and the like.

[0033] Brain function disorder includes psychotic condition due to cerebral hemorrhage, cerebral thrombus, cerebral embolus, subarachnoid hemorrhage, transient cerebral ischemic stroke, hypertensive encephalopathy, cerebral arteriosclerosis, subdural hematoma, extradural hematoma, cerebral hypoxia, cerebral edema, cerebritis, cerebral tumor, external injury in head, mental disease, metabolite poisoning, drug poisoning, temporal respiratory arrest, deep anesthesia during operation, physical disorder and the like, and sequelae, decreased attention, hyperactivity, logopathy, delayed mental development, lethe, dementia (inclusive of wandering, nocturnal delirium, aggressive behavior and the like associated with dementia) caused by the above-mentioned diseases.

[0034] Therefore, the Rho kinase inhibitor of the present invention is effective as a pharmaceutical agent, particularly as an agent for the prophylaxis and treatment of these diseases caused by Rho, such as a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a prophylactic agent of immature birth, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a contraceptive, a prophylactic agent of digestive tract infection, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy and a brain function improving drug.

[0035] The compounds of the formula (I) and the formula (II) have high affinity for Rho kinase. Thus, the radioactive substance (radio ligand) thereof are industrially useful as a selective radio ligand of Rho kinase. The compounds of the formula (I) and the formula (II) and modified compounds thereof (e.g., radio ligand of these compounds and the like), which are Rho kinase inhibitors, are useful as reagents for the study of Rho and Rho kinase and as diagnostics of the diseases relating to them.

[0036] The compound to be used as the Rho kinase inhibitor of the present invention may be any as long as it has a

Rho kinase inhibitory action. For example, the compounds of the formula (I) and the formula (II) are used.

[0037] In the present specification, each symbol of the formula (I) is defined as follows.

[0038] Alkyl at R and R^1 is linear or branched alkyl having 1 to 10 carbon atoms, which is exemplified by methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl and the like, with preference given to alkyl having 1 to 4 carbon atoms.

[0039] Cycloalkyl at R and R^1 has 3 to 7 carbon atoms and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like.

[0040] Cycloalkylalkyl at R and R^1 is that wherein the cycloalkyl moiety is the above-mentioned cycloalkyl having 3 to 7 carbon atoms and the alkyl moiety is linear or branched alkyl having 1 to 6 carbon atoms (e.g., methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl and the like), which is exemplified by cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, cycloheptylmethyl, cyclopropylethyl, cyclopentylethyl, cyclohexylethyl, cycloheptylethyl, cyclopropylpropyl, cyclopentylpropyl, cyclohexylpropyl, cycloheptylpropyl, cyclopropylbutyl, cyclopentylbutyl, cyclohexylbutyl, cycloheptylbutyl, cyclopropylhexyl, cyclopentylhexyl, cyclohexylhexyl, cycloheptylhexyl and the like.

[0041] Aralkyl at R and R^1 is that wherein alkyl moiety is alkyl having 1 to 4 carbon atoms and is exemplified by phenylalkyl such as benzyl, 1-phenylethyl, 2-phenylethyl, 3-phenylpropyl, 4-phenylbutyl and the like.

[0042] The substituent of optionally substituted cycloalkyl, cycloalkylalkyl, phenyl and aralkyl on the ring at R and R^1 is halogen (e.g., chlorine, bromine, fluorine and iodine), alkyl (same as alkyl at R and R^1), alkoxy (linear or branched alkoxy having 1 to 6 carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, tert-butoxy, pentyloxy, hexyloxy and the like), aralkyl (same as aralkyl at R and R^1) or haloalkyl (alkyl at R and R^1 which is substituted by 1-5 halogen, and exemplified by fluoromethyl, difluoromethyl, trifluoromethyl, 2,2,2-trifluoroethyl, 2,2,3,3,3-pentafluoropropyl and the like), nitro, amino, cyano, azide and the like.

[0043] The group formed by R and R^1 in combination together with the adjacent nitrogen atom, which forms a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom is preferably a 5 or 6-membered ring and bonded ring thereof. Examples thereof include 1-pyrrolidinyl, piperidino, 1-piperazinyl, morpholino, thiomorpholino, 1-imidazolyl, 2,3-dihydrothiazol-3-yl and the like. The substituent of the optionally substituted nitrogen atom is exemplified by alkyl, aralkyl, haloalkyl and the like. As used herein, alkyl, aralkyl and haloalkyl are as defined for R and R^1.

[0044] Alkyl at R^2 is as defined for R and R^1.

[0045] Halogen, alkyl, alkoxy and aralkyl at R^3 and R^4 are as defined for R and R^1.

[0046] Acyl at R^3 and R^4 is alkanoyl having 2 to 6 carbon atoms (e.g., acetyl, propionyl, butyryl, valeryl, pivaloyl and the like), benzoyl or phenylalkanoyl wherein the alkanoyl moiety has 2 to 4 carbon atoms (e.g., phenylacetyl, phenylpropionyl, phenylbutyryl and the like).

[0047] Alkylamino at R^3 and R^4 is that wherein the alkyl moiety is alkylamino having linear or branched alkyl having 1 to 6 carbon atoms. Examples thereof include methylamino, ethylamino, propylamino, isopropylamino, butylamino, isobutylamino, sec-butylamino, tert-butylamino, pentylamino, hexylamino and the like.

[0048] Acylamino at R^3 and R^4 is that wherein acyl moiety is alkanoyl having 2 to 6 carbon atoms, benzyl or the alkanoyl moiety is phenylalkanoyl having 2 to 4 carbon atoms and the like, which is exemplified by acetylamino, propionylamino, butyrylamino, valerylamino, pivaloylamino, benzoylamino, phenylacetylamino, phenylpropionylamino, phenylbutyrylamino and the like.

[0049] Alkylthio at R^3 and R^4 is that wherein the alkyl moiety is linear or branched alkyl having 1 to 6 carbon atoms, which is exemplified by methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, sec-butylthio, tert-butylthio, pentylthio, hexylthio and the like.

[0050] Aralkyloxy at R^3 and R^4 is that wherein the alkyl moiety is alkyl having 1 to 4 carbon atoms, which is exemplified by benzyloxy, 1-phenylethyloxy, 2-phenylethyloxy, 3-phenylpropyloxy, 4-phenylbutyloxy and the like.

[0051] Aralkylthio at R^3 and R^4 is that wherein the alkyl moiety is alkyl having 1 to 4 carbon atoms, which is exemplified by benzylthio,1-phenylethylthio,2-phenylethylthio,3-phenylpropylthio,4-phenylbutylthio and the like.

[0052] Alkoxycarbonyl at R^3 and R^4 is that wherein the alkoxy moiety is linear or branched alkoxy having 1 to 6 carbon atoms, which is exemplified by methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, sec-butoxycarbonyl, tert-butoxycarbonyl, pentyloxycarbonyl, hexyloxycarbonyl and the like.

[0053] Alkylcarbamoyl at R^3 and R^4 is carbamoyl mono- or di-substituted by alkyl having 1 to 4 carbon atoms, which is exemplified by methylcarbamoyl, dimethylcarbamoyl, ethylcarbamoyl, diethylcarbamoyl, propylcarbamoyl, dipropylcarbamoyl, butylcarbamoyl, dibutylcarbamoyl and the like.

[0054] Alkoxy at R^5 is as defined for R and R^1.

[0055] Alkoxycarbonyloxy at R^5 is that wherein the alkoxy moiety is linear or branched alkoxy having 1 to 6 carbon atoms, which is exemplified by methoxycarbonyloxy, ethoxycarbonyloxy, propoxycarbonyloxy, isopropoxycarbonyloxy, butoxycarbonyloxy, isobutoxycarbonyloxy, sec-butoxycarbonyloxy, tert-butoxycarbonyloxy, pentyloxycarbonyloxy, hexyloxycarbonyloxy and the like.

[0056] Alkanoyloxy at R^5 is that wherein the alkanoyl moiety is alkanoyl having 2 to 6 carbon atoms, which is exem-

plified by acetyloxy, propionyloxy, butyryloxy, valeryloxy, pivaloyloxy and the like.

[0057] Aralkyloxycarbonyloxy at R^5 is that wherein the aralkyl moiety is aralkyl having C_1-C_4 alkyl, which is exemplified by benzyloxycarbonyloxy, 1-phenylethyloxycarbonyloxy, 2-phenylethyloxycarbonyloxy, 3-phenylpropyloxycarbonyloxy, 4-phenylbutyloxycarbonyloxy and the like.

[0058] Alkyl at R^6 is as defined for R and R^1; alkyl at R^8 and R^9 is as defined for R and R^1; and aralkyl at R^8 and R^9 is as defined for R and R^1.

[0059] Alkyl at R^7 is as defined for R and R^1 and aralkyl at R^7 is as defined for R and R^1.

[0060] The group formed by R^6 and R^7 in combination, which forms a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom, is imidazol-2-yl, thiazol-2-yl, oxazol-2-yl, imidazolin-2-yl, 3,4,5,6-tetrahydropyridin-2-yl, 3,4,5,6-tetrahydropyrimidin-2-yl, 1,3-oxazolin-2-yl, 1,3-thiazolin-2-yl or optionally substituted benzoimidazol-2-yl, benzothiazol-2-yl, benzoxazol-2-yl and the like having a substituent such as halogen, alkyl, alkoxy, haloalkyl, nitro, amino, phenyl, aralkyl and the like. As used herein, halogen, alkyl, alkoxy, haloalkyl and aralkyl are as defined for R and R^1.

[0061] The substituent of the above-mentioned optionally substituted nitrogen atom is exemplified by alkyl, aralkyl, haloalkyl and the like. As used herein, alkyl, aralkyl and haloalkyl are as defined for R and R^1.

[0062] Hydroxyalkyl at R^{10} and R^{11} is linear or branched alkyl having 1 to 6 carbon atoms which is substituted by 1 to 3 hydroxy, which is exemplified by hydroxymethyl, 2-hydroxyethyl, 1-hydroxyethyl, 3-hydroxypropyl, 4-hydroxybutyl and the like. Alkyl at R^{10} and R^{11} is as defined for R and R^1; haloalkyl and alkoxycarbonyl at R^{10} and R^{11} are as defined for R and R^1 ; aralkyl at R^{10} and R^{11} is as defined for R and R^1; and cycloalkyl formed by R^{10} and R^{11} in combination is the same as cycloalkyl at R and R^1.

[0063] Alkyl at L is as defined for R and R^1.

[0064] Aminoalky at L is a linear or branched alkyl having 1 to 6 carbon atoms, which is substituted by amino, which is exemplified by aminomethyl, 2-aminoethyl, 1-aminoethyl, 3-aminopropyl, 4-aminobutyl, 5-aminopentyl, 6-aminohexyl and the like.

[0065] Mono- or dialkylaminoalkyl at L is mono- or di-substituted aminoalkyl with alkyl having 1 to 4 carbon atoms, which is exemplified by methylaminomethyl, dimethylaminomethyl, ethylaminomethyl, diethylaminomethyl, propylaminomethyl, dipropylaminomethyl, butylaminomethyl, dibutylaminomethyl, 2-dimethylaminoethyl, 2-diethylaminoethyl and the like.

[0066] Carbamoylalkyl at L is linear or branched alkyl having 1 to 6 carbon atoms substituted by carbamoyl, which is exemplified by carbamoylmethyl, 2-carbamoylethyl, 1-carbamoylethyl, 3-carbamoylpropyl, 4-carbamoylbutyl, 5-carbamoylpentyl, 6-carbamoylhexyl and the like.

[0067] Phthalimidoalkyl at L is linear or branched alkyl having 1 to 6 carbon atoms, which is substituted by phthalimide. Examples thereof include phthalimidomethyl, 2-phthalimidoethyl, 1-phthalimidoethyl, 3-phthalimidopropyl, 4-phthalimidobutyl, 5-phthalimidopentyl, 6-phthalimidohexyl and the like.

[0068] Alkyl at B is as defined for R and R^1.

[0069] Alkoxy at B is as defined for R and R^1.

[0070] Aralkyl at B is as defined for R and R^1.

[0071] Aralkyloxy at B is as defined for R^3 and R^4.

[0072] Aminoalkyl at B is as defined for L.

[0073] Hydroxyalkyl at B is as defined for R^{10} and R^{11}.

[0074] Alkanoyloxyalkyl at B is that wherein linear or branched alkyl having 1 to 6 carbon atoms is substituted by alkanoyloxy having alkanoyl moiety having 2 to 6 carbon atoms, which is exemplified by acetyloxymethyl, propionyloxymethyl, butyryloxymethyl, valeryloxymethyl, pivaloyloxymethyl, acetyloxyethyl, propionyloxyethyl, butyryloxyethyl, valeryloxyethyl, pivaloyloxyethyl and the like.

[0075] Alkoxycarbonylalkyl at B is that wherein linear or branched alkyl having 1 to 6 carbon atoms is substituted by alkoxycarbonyl having alkoxy moiety having 1 to 6 carbon atoms, which is exemplified by methoxycarbonylmethyl, ethoxycarbonylmethyl, propoxycarbonylmethyl, isopropoxycarbonylmethyl, butoxycarbonylmethyl, isobutoxycarbonylmethyl, sec-butoxycarbonylmethyl, tert-butoxycarbonylmethyl, pentyloxycarbonylmethyl, hexyloxycarbonylmethyl, methoxycarbonylethyl, ethoxycarbonylethyl, propoxycarbonylethyl, isopropoxycarbonylethyl, butoxycarbonylethyl, isobutoxycarbonylethyl, sec-butoxycarbonylethyl, tert-butoxycarbonylethyl, pentyloxycarbonylethyl, hexyloxycarbonylethyl and the like.

[0076] Halogen at Q^1, Q^2 and Q^3 is as defined for R and R^1.

[0077] Aralkyloxy at Q^1 and Q^2 is as defined for R^3 and R^4.

[0078] Alkoxy at Q^3 is as defined for R and R^1.

[0079] Alkylene at W, X and Y is linear or branched alkylene having 1 to 6 carbon atoms, which is exemplified by methylene, ethylene, trimethylene, propylene, tetramethylene, pentamethylene, hexamethylene and the like.

[0080] Alkenylene at Y is linear or branched alkenylene having 2 to 6 carbon atoms, which is exemplified by vinylene, propenylene, butenylene, pentenylene and the like.

[0081] Alkyl at Rb is as defined for R and R^1.

[0082] Aralkyl at Rb is as defined for R and R^1.

[0083] Aminoalkyl at Rb is as defined for L.

[0084] Mono- or dialkylaminoalkyl at Rb is as defined for L.

[0085] The heterocycle when single ring containing nitrogen at Rc is pyridine, pyrimidine, pyridazine, triazine, pyrazole, triazole and the like, and when it is a condensed ring, it is exemplified by pyrrolopyridine (e.g., 1H-pyrrolo[2,3-b]pyridine, 1H-pyrrolo[3,2-b]pyridine, 1H-pyrrolo[3,4-b]pyridine and the like), pyrazolopyridine (e.g., 1H-pyrazolo[3,4-b]pyridine, 1H-pyrazolo[4,3-b]pyridine and the like), imidazopyridine (e.g., 1H-imidazo[4,5-b]pyridine and the like), pyrrolopyrimidine (e.g., 1H-pyrrolo[2,3-d]pyrimidine, 1H-pyrrolo[3,2-d]pyrimidine, 1H-pyrrolo[3,4-d]pyrimidine and the like), pyrazolopyrimidine (e.g., 1H-pyrazolo[3,4-d]pyrimidine, pyrazolo[1,5-a]pyrimidine, 1H-pyrazolo[4,3-d]pyrimidine and the like), imidazopyrimidine (e.g., imidazo[1,2-a]pyrimidine, 1H-imidazo[4,5-d]pyrimidine and the like), pyrrolotriazine (e.g., pyrrolo[1,2-a]-1,3,5-triazine, pyrrolo[2,1-f]-1,2,4-triazine), pyrazolotriazine (e.g., pyrazolo[1,5-a]-1,3,5-triazine and the like), triazolopyridine (e.g., 1H-1,2,3-triazolo[4,5-b]pyridine and the like), triazolopyrimidine (e.g., 1,2,4-triazolo[1,5-a]pyrimidine, 1,2,4-triazolo[4,3-a]pyrimidine, 1H-1,2,3-triazolo[4,5-d]pyrimidine and the like), cinnoline, quinazoline, quinoline, pyridopyridazine (e.g., pyrido[2,3-c]pyridazine and the like), pyridopyrazine (e.g., pyrido[2,3-b]pyrazine and the like), pyridopyrimidine (e.g., pyrido[2,3-d]pyrimidine, pyrido[3,2-d]pyrimidine and the like), pyrimidopyrimidine (e.g., pyrimido[4,5-d]pyrimidine, pyrimido[5,4-d]pyrimidine and the like), pyrazinopyrimidine (e.g., pyrazino[2,3-d]pyrimidine and the like), naphthyridine (e.g., 1,8-naphthyridine and the like), tetrazolopyrimidine (e.g., tetrazolo[1,5-a]pyrimidine and the like), thienopyridine (e.g., thieno[2,3-b]pyridine and the like), thienopyrimidine (e.g., thieno[2,3-d]pyrimidine and the like), thiazolopyridine (e.g., thiazolo[4,5-b]pyridine, thiazolo[5,4-b]pyridine and the like), thiazolopyrimidine (e.g., thiazolo[4,5-d]pyrimidine, thiazolo[5,4-d]pyrimidine and the like), oxazolopyridine (e.g., oxazolo[4,5-b]pyridine, oxazolo[5,4-b]pyridine and the like), oxazolopyrimidine (e.g., oxazolo[4,5-d]pyrimidine, oxazolo[5,4-d]pyrimidine and the like), furopyridine (e.g., furo[2,3-b]pyridine, furo[3,2-b]pyridine and the like), furopyrimidine (e.g., furo[2,3-d]pyrimidine, furo[3,2-d]pyrimidine and the like), 2,3-dihydropyrrolopyridine (e.g., 2,3-dihydro-1H-pyrrolo[2,3-b]pyridine, 2,3-dihydro-1H-pyrrolo[3,2-b]pyridine and the like), 2,3-dihydropyrrolopyrimidine (e.g., 2,3-dihydro-1H-pyrrolo[2,3-d]pyrimidine, 2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidine and the like), 5,6,7,8-tetrahydropyrido[2,3-d]pyrimidine, 5,6,7,8-tetrahydro-1,8-naphthyridine, 5,6,7,8-tetrahydroquinoline and the like. When these rings form a hydrogenated aromatic ring, the carbon atom in the ring may be carbonyl and includes, for example, 2,3-dihydro-2-oxopyrrolopyridine, 2,3-dihydro-2,3-dioxopyrrolopyridine, 7,8-dihydro-7-oxo-1,8-naphthyridine, 5,6,7,8-tetrahydro-7-oxo-1,8-naphthyridine and the like.

[0086] These rings may be substituted by a substituent such as halogen, alkyl, alkoxy, aralkyl, haloalkyl, nitro, amino, alkylamino, cyano, formyl, acyl, aminoalkyl, mono- or dialkylaminoalkyl, azide, carboxy, alkoxycarbonyl, carbamoyl, alkylcarbamoyl, alkoxyalkyl (e.g., methoxymethyl, methoxyethyl, methoxypropyl, ethoxymethyl, ethoxyethyl, ethoxypropyl and the like), optionally substituted hydrazino and the like.

[0087] As used herein, the substituent of the optionally substituted hydrazino includes alkyl, aralkyl, nitro, cyano and the like, wherein alkyl and aralkyl are as defined for R and R^1 and exemplified by methyl hydrazino, ethyl hydrazino, benzyl hydrazino and the like.

[0088] In the present specification, each symbol of the formula (II) is defined as follows.

[0089] The linear or branched alkyl having 1 to 6 carbon atoms at R^{13}, R^{14}, R^{15} and R^{16} is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

[0090] Axyl at R^{14} and R^{15} is phenyl, naphthyl and the like.

[0091] Aralkyl at R^{14} and R^{15} is as defined for R and R^1.

[0092] Alkylene having 4 or less carbon atoms, which is formed by R^{13} and R^{14} directly bonded to each other, is methylene, ethylene, trimethylene, propylene, tetramethylene and the like.

[0093] Alkyl having 1 to 10 carbon atoms, which substitutes alkylene having 4 or less carbon atoms formed by R^{13} and R^{14} directly bonded to each other, is linear or branched alkyl having 1 to 10 carbon atoms. Examples thereof include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl and the like.

[0094] Alkyl having 1 to 6 carbon atoms which substitutes ethylene and trimethylene formed by R^{13} and R^{14} directly bonded to each other is linear or branched alkyl having 1 to 6 carbon atoms, which is the same as those for R^{13}.

[0095] The heterocycle formed by R^{14} and R^{15} directly or via oxygen atom bonded together with the adjacent nitrogen atom is pyrrolidino, piperidino, morpholino, homopiperidino, homomorpholino and the like.

[0096] Alkylene having 2 to 4 carbon atoms formed by R^{17} and R^{18} directly bonded to each other is ethylene, trimethylene, propylene, tetramethylene and the like.

[0097] Alkylene having 2 to 6 carbon atoms at Alk is ethylene, trimethylene, propylene, tetramethylene, pentamethylene, hexamethylene and the like.

[0098] Alkyl having 1 to 6 carbon atoms and alkyl having 1 to 10 carbon atoms, which are the substituents of alkylene having 2 to 6 carbon atoms at Alk, are as defined for R^{13}.

[0099] Aryl and aralkyl, which are the substituents of alkylene having 2 to 6 carbon atoms at Alk, are as defined for

R^{14}.

[0100] The compound to be used as the Rho kinase inhibitor of the present invention is, for example, a compound of the formula (I), which is exemplified by the following compounds.

(1) 4-(2-pyridylcarbamoyl)piperidine
(2) 1-benzyloxycarbonyl-4-(4-pyridylcarbamoyl)piperidine
(3) 1-benzoyl-4-(4-pyridylcarbamoyl)piperidine
(4) 1-propyl-4-(4-pyridylcarbamoyl)piperidine
(5) [3-(2-(2-thienylmethyl)phenoxy)-2-hydroxypropyl]-4-(4-pyridylcarbamoyl)piperidine
(6) 4-(4-pyridylcarbamoyl)piperidine
(7) 1-benzyl-4-(4-pyridylcarbamoyl)-1,2,5,6-tetrahydropyridine
(8) 3-(4-pyridylcarbamoyl)piperidine
(9) 1-benzyl-3-(4-pyridylcarbamoyl)piperidine
(10) 1-(2-(4-benzyloxyphenoxy)ethyl)-4-(N-(2-pyridyl)-N-benzylcarbamoyl)pyridine
(11) 1-formyl-4-(4-pyridylcarbamoyl)piperidine
(12) 4-(3-pyridylcarbamoyl)piperidine
(13) 1-isopropyl-4-(4-pyridylcarbamoyl)piperidine
(14) 1-methyl-4-(4-pyridylcarbamoyl)piperidine
(15) 1-hexyl-4-(4-pyridylcarbamoyl)piperidine
(16) 1-benzyl-4-(4-pyridylcarbamoyl)piperidine
(17) 1-(2-phenylethyl)-4-(4-pyridylcarbamoyl)piperidine
(18) 1-(2-(4-methoxyphenyl)ethyl)-4-(4-pyridylcarbamoyl)piperidine
(19) 1-(2-(4-methoxyphenyl)ethyl)-4-(2-pyridylcarbamoyl)piperidine
(20) 1-(2-(4-chlorophenyl)ethyl)-4-(4-pyridylcarbamoyl)piperidine
(21) 1-diphenylmethyl-4-(2-pyridylcarbamoyl)piperidine
(22) 1-[2-(4-(5-methyl-3-oxo-2,3,4,5-tetrahydropyridazin-6-yl)phenyl)ethyl]-4-(2-pyridylcarbamoyl)piperidine
(23) 1-(4-(4,5-dihydro-2-furyl)phenyl)-4-(4-pyridylcarbamoyl)piperidine
(24) 1-(2-nitrophenyl)-4-(4-pyridylcarbamoyl)piperidine
(25) 1-(2-aminophenyl)-4-(4-pyridylcarbamoyl)piperidine
(26) 1-nicotinoyl-4-(4-pyridylcarbamoyl)piperidine
(27) 1-isonicotinoyl-4-(4-pyridylcarbamoyl)piperidine
(28) 1-(3,4,5-trimethoxybenzoyl)-4-(4-pyridylcarbamoyl)piperidine
(29) 1-acetyl-4-(4-pyridylcarbamoyl)piperidine
(30) 1-(3-(4-fluorobenzoyl)propyl)-4-(4-pyridylcarbamoyl)piperidine
(31) 1-(3-(4-fluorobenzoyl)propyl)-4-(2-pyridylcarbamoyl)piperidine
(32) 1-(1-(4-hydroxybenzoyl)ethyl)-4-(2-pyridylcarbamoyl)piperidine
(33) 1-(1-(4-benzyloxybenzoyl)ethyl)-4-(2-pyridylcarbamoyl)piperidine
(34) 1-(2-(4-hydroxphenoxy)ethyl)-4-(2-pyridylcarbamoyl)piperidine
(35) 1-(4-(4-fluorophenyl)-4-hydroxybutyl)-4-(4-pyridylcarbamoyl)piperidine
(36) 1-(1-methyl-2-(4-hydroxyphenyl)-2-hydroxyethyl)-4-(2-pyridylcarbamoyl)piperidine
(37) 1-cinnamyl-4-(2-pyridylcarbamoyl)piperidine
(38) 1-(2-hydroxy-3-phenoxypropyl)-4-(4-pyridylcarbamoyl)piperidine
(39) 1-(2-hydroxy-3-phenoxypropyl)-4-(3-pyridylcarbamoyl)piperidine
(40) 1-(2-hydroxy-3-phenoxypropyl)-4-(2-pyridylcarbamoyl)piperidine
(41) 1-(2-phenylethyl)-4-[N-(2-pyridyl)-N-(2-(N, N-dimethylamino)ethyl)carbamoyl]piperidine
(42) 1-benzyloxycarbonyl-4-(2-pyridylcarbamoyl)piperidine
(43) 1-(3-chlorophenyl)carbamoyl-4-(4-pyridylcarbamoyl)piperidine
(44) 1-[N-(2-pyridyl)-N-(2-(N, N-dimethylamino)ethyl)carbamoyl]piperidine
(45) 1-methyl-4-(4-pyridylcarbamoyl)-1,2,5,6-tetrahydropyridine
(46) 1-nicotinoyl-3-(4-pyridylcarbamoyl)piperidine
(47) 1-[2-(4-fluorobenzoyl)ethyl]-4-(4-pyridylcarbamoyl)piperidine
(48) 1-(6-chloro-2-methylimidazo[1,2-a]pyridine-3-carbonyl)-4-(4-pyridylcarbamoyl)piperidine
(49) 1-(4-nitrobenzyl)-4-(4-pyridylcarbamoyl)piperidine
(50) 1-hexyl-4-(4-pyridylcarbamoyl)piperidine
(51) 1-benzyloxycarbonyl-4-(2-chloro-4-pyridylcarbamoyl)piperidine
(52) 4-(2-chloro-4-pyridylcarbamoyl)piperidine
(53) 1-(2-chloronicotinoyl)-4-(4-pyridylcarbamoyl)piperidine
(54) 3-(2-chloro-4-pyridylcarbamoyl)piperidine

(55) 1-(4-phthalimidobutyl)-4-(4-pyridylcarbamoyl)piperidine
(56) 1-(3,5-di-tert-butyl-4-hydroxycinnamoyl)-4-(4-pyridylcarbamoyl)piperidine
(57) 1-carbamoylmethyl-4-(4-pyridylcarbamoyl)piperidine
(58) 1-benzyloxycarbonyl-4-(5-nitro-2-pyridylcarbamoyl)piperidine
(59) 4-(5-nitro-2-pyridylcarbamoyl)piperidine
(60) trans-4-benzyloxycarboxamidomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(61) trans-4-aminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(62) trans-4-formamidomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(63) trans-4-dimethylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(64) N-benzylidene-trans-(4-pyridylcarbamoyl)cyclohexylmethylamine
(65) trans-4-benzylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(66) trans-4-isopropylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(67) trans-4-nicotinoylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(68) trans-4-cyclohexylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(69) trans-4-benzyloxycarboxamide-1-(4-pyridylcarbamoyl)cyclohexane
(70) trans-4-amino-1-(4-pyridylcarbamoyl)cyclohexane
(71) trans-4-(1-aminoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(72) trans-4-aminomethyl-cis-2-methyl-1-(4-pyridylcarbamoyl)cyclohexane
(73) (+)-trans-4-(1-benzyloxycarboxamidopropyl)-1-cyclohexanecarboxylic acid
(74) (+)-trans-4-(1-benzyloxycarboxamidopropyl)-1-(4-pyridylcarbamoyl)cyclohexane
(75) (-)-trans-4-(1-benzyloxycarboxamidpropyl)-1-(4-pyridylcarbamoyl)cyclohexane
(76) (+)-trans-4-(1-aminopropyl)-1-(4-pyridylcarbamoyl)cyclohexane
(77) (-)-trans-4-(1-aminopropyl)-1-(4-pyridylcarbamoyl)cyclohexane
(78) (-)-trans-4-(1-benzyloxycarboxamidoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(79) (+)-trans-4-(1-benzyloxycarboxamidoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(80) (+)-trans-4-(1-aminoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(81) (-)-trans-4-(1-aminoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(82) trans-4-(4-chlorobenzoyl)aminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(83) trans-4-aminomethyl-1-(2-pyridylcarbamoyl)cyclohexane
(84) trans-4-benzyloxycarboxamidomethyl-1-(2-pyridylcarbamoyl)cyclohexane
(85) trans-4-methylaminomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(86) trans-4-(N-benzyl-N-methylamino)methyl-1-(4-pyridylcarbamoyl)cyclohexane
(87) trans-4-aminomethyl-1-(3-pyridylcarbamoyl)cyclohexane
(88) trans-4-aminomethyl-1-[(3-hydroxy-2-pyridyl)carbamoyl]cyclohexane
(89) trans-4-benzyloxycarboxamidomethyl-1-(3-pyridylcarbamoyl)cyclohexane
(90) trans-4-benzyloxycarboxamidomethyl-1-[(3-benzyloxy-2-pyridyl)carbamoyl]cyclohexane
(91) trans-4-phthalimidomethyl-1-(4-pyridylcarbamoyl)cyclohexane
(92) trans-4-benzyloxycarboxamidomethyl-1-(3-methyl-4-pyridylcarbamoyl)cyclohexane
(93) trans-4-aminomethyl-1-(3-methyl-4-pyridylcarbamoyl)cyclohexane
(94) 4-(trans-4-benzyloxycarboxamidomethylcyclohexylcarbonyl)amino-2,6-dimethylpyridine-N-oxide
(95) 4-(trans-4-aminomethylcyclohexylcarbonyl)amino-2,6-dimethylpyridine-N-oxide
(96) trans-4-aminomethyl-1-(2-methyl-4-pyridylcarbamoyl)cyclohexane
(97) trans-4-(1-benzyloxycarboxamidoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(98) trans-4-(1-amino-1-methylethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(99) trans-4-(2-aminoethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(100) trans-4-(2-amino-1-methylethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(101) trans-4-(1-aminopropyl)-1-(4-pridylcarbamoyl)cyclohexane
(102) trans-4-aminomethyl-trans-1-methyl-1-(4-pyridylcarbamoyl)cyclohexane
(103) trans-4-benzylaminomethyl-cis-2-methyl-1-(4-pyridylcarbamoyl)cyclohexane
(104) trans-4-(1-benzyloxycarboxamide-1-methylethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(105) trans-4-benzyloxycarboxamidomethyl-1-(N-methyl-4-pyridylcarbamoyl)cyclohexane
(106) trans-4-(1-acetamide-1-methylethyl)-1-(4-pyridylcarbamoyl)cyclohexane
(107) trans-N-(6-amino-4-pyrimidyl)-4-aminomethylcyclohexanecarboxamide
(108) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(109) (+)-trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide
(110) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(111) trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(112) (+)-trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide

(113) trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(114) (+)-trans-N-(2-amino-4-pyridyl)-4-(1-aminoethyl)cyclohexanecarboxamide
(115) trans-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)-4-aminomethylcyclohexanecarboxamide
(116) (+)-trans-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide
(117) trans-N-(1H-pyrazolo[3,4-d]pyrimidin-4-yl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(118) trans-N-(4-pyrimidinyl)-4-aminomethylcyclohexanecarboxamide
(119) trans-N-(3-amino-4-pyridyl)-4-aminomethylcyclohexanecarboxamide
(120) trans-N-(7H-imidazo[4,5-d]pyrimidin-6-yl)-4-aminomethylcyclohexanecarboxamide
(121) trans-N-(3H-1,2,3-triazolo[4,5-d]pyrimidin-7-yl)-4-aminomethylcyclohexanecarboxamide
(122) trans-N-(1-benzyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(123) trans-N-(1H-5-pyrazolyl)-4-aminomethylcyclohexanecarboxamide
(124) trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(125) trans-N-(4-pyridazinyl)-4-aminomethylcyclohexanecarboxamide
(126) trans-N-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-4-aminomethylcyclohexanecarboxamide
(127) trans-N-(2-amino-4-pyridyl)-4-aminomethylcyclohexanecarboxamide
(128) trans-N-(thieno[2,3-d]pyrimidin-4-yl)-4-aminomethylcyclohexanecarboxamide
(129) trans-N-(5-methyl-1,2,4-triazolo[1,5-a]pyrimidin-7-yl)-4-aminomethylcyclohexanecarboxamide
(130) trans-N-(3-cyano-5-methylpyrazolo[1,5-a]pyrimidin-7-yl)-4-aminomethylcyclohexanecarboxamide
(131) trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(132) trans-N-(2-(1-pyrrolidinyl)-4-pyridyl)-4-aminomethylcyclohexanecarboxamide
(133) trans-N-(2,6-diamino-4-pyrimidyl)-4-aminomethylcyclohexanecarboxamide
(134) (+)-trans-N-(7-methyl-1,8-naphthyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide
(135) trans-N-(1-benzyloxymethylpyrrolo[2,3-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(136) (+)-trans-N-(1-methylpyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide
(137) trans-N-benzyl-N-(2-benzylamino-4-pyridyl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(138) trans-N-(2-azide-4-pyridyl)-4-aminomethylcyclohexanecarboxamide
(139) trans-N-(2,3-dihydro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-aminomethylcyclohexanecarboxamide
(140) trans-N-(2,3-dihydro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-amino-1-methylethyl)cyclohexanecarboxamide
(141-1) trans-N-(2-carboxy-4-pyridyl)-4-aminomethylcyclohexanecarboxamide
(141-2) (R)-(+)-trans-N-(3-bromo-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)cyclohexanecarboxamide
(142) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-guanidinomethylcyclohexanecarboxamide
(143) trans-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-guanidinomethylcyclohexanecarboxamide
(144) trans-N-(4-pyridyl)-4-guanidinomethylcyclohexanecarboxamide
(145) trans-N-(1-methylpyrrolo[2,3-b]pyridin-4-yl)-4-(guanidinomethyl)cyclohexanecarboxamide
(146) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(2-imidazolin-2-yl)aminomethylcyclohexanecarboxamide
(147) trans-N-(1-benzyloxymethylpyrrolo[2,3-b]pyridin-4-yl)-4-guanidinomethylcyclohexanecarboxamide
(148) trans-N-(2-amino-4-pyridyl)-4-guanidinomethylcyclohexanecarboxamide
(149) trans-N-(1-benzyloxymethyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(2-imidazolin-2-yl)aminomethylcyclohexanecarboxamide
(150) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(3-benzylguanidinomethyl)cyclohexanecarboxamide
(151) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(3-phenylguanidinomethyl)cyclohexanecarboxamide
(152) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(3-propylguanidinomethyl)cyclohexanecarboxamide
(153) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(3-octylguanidinomethyl)cyclohexanecarboxamide
(154) trans-N-(1-benzyloxymethylpyrrolo[2,3-b]pyridin-4-yl)-4-(2-benzyl-3-ethylguanidinomethyl)cyclohexanecarboxamide
(155) trans-N-(1H-pyrrolo[2,3-b]pyridin 4 yl)-4-(imidazol-2-yl)aminomethylcyclohexanecarboxamide
(156) trans-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(thiazol-2-yl)aminomethylcyclohexanecarboxamide
(157) (R)-(+)-N-(4-pyridyl)-4-(1-aminoethyl)benzamide
(158) N-(4-pyridyl)-4-(1-amino-1-methylethyl)benzamide
(159) N-(4-pyridyl)-4-aminomethyl-2-benzyloxybenzamide
(160) N-(4-pyridyl)-4-aminomethyl-2-ethoxybenzamide
(161) (R)-(-)-N-(4-pyridyl)-4-(1-aminoethyl)-3-nitrobenzamide
(162) (R)-(-)-N-(4-pyridyl)-3-amino-4-(1-aminoethyl)benzamide
(163) (R)-(+)-N-(4-pyridyl)-4-(1-aminoethyl)-3-chlorobenzamide
(164) N-(4-pyridyl)-3-aminomethylbenzamide
(165) (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide
(166) (R)-(+)-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide
(167) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-guanidinomethylbenzamide

(168) N-(4-pyridyl)-4-guanidinomethylbenzamide
(169) (R)-(+)-N-(4-pyridyl)-4-(1-aminoethyl)-3-fluorobenzamide
(170) N-(4-pyridyl)-4-aminomethylbenzamide
(171) N-(4-pyridyl)-4-aminomethyl-2-hydroxybenzamide
(172) N-(4-pyridyl)-4-(2-aminoethyl)benzamide
(173) N-(4-pyridyl)-4-aminomethyl-3-nitrobenzamide
(174) N-(4-pyridyl)-3-amino-4-aminomethylbenzamide
(175) (S)-(-)-N-(4-pyridyl)-4-(1-aminoethyl)benzamide
(176) (S)-(-)-N-(4-pyridyl)-2-(1-aminoethyl)benzamide
(177) (R)-(+)-N-(4-pyridyl)-4-(1-aminoethyl)-2-chlorobenzamide
(178) (R)-(+)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-(3-propylguanidino)ethyl)benzamide
(179) (R)-(-)-N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-3-azidebenzamide
(180) (R)-(+)-N-(4-pyridyl)-4-(1-aminoethyl)-2-nitrobenzamide
(181) (R)-(-)-N-(4-pyridyl)-4-(1-aminoethyl)-3-ethoxybenzamide
(182) (R)-(+)-N-(3-iodo-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)benzamide
(183) (R)-(+)-N-(3-iodo-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-(1-aminoethyl)-3-azidebenzamide
(184) (R)-(-)-N-(4-pyridyl)-4-(1-aminoethyl)-3-hydroxybenzamide
(185) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-guanidinomethyl-3-nitrobenzamide
(186) (R)-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-guanidinoethyl)-3-nitrobenzamide
(187) (R)-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-aminoethyl)-2-nitrobenzamide
(188) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-guanidinobenzamide
(189) (R)-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-aminoethyl)-3-nitrobenzamide
(190) (R)-N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-guanidinoethyl)benzamide
(191) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-(1-amino-2-hydroxyethyl)benzamide
(192) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-aminomethyl-3-nitrobenzamide
(193) N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide
(194) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-piperidinecarboxamide
(195) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-aminoacetyl-4-piperidinecarboxamide
(196) N-(1-methoxymethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-4-piperidinecarboxamide
(197) N-(2,3-dihydro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide
(198) N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide
(199) N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-amidino-4-piperidinecarboxamide
(200) N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(3-phenylpropyl)-4-piperidinecarboxamide
(201) N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyl-4-piperidinecarboxamide
(202) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide
(203) N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-(3-phenylpropyl)-4-piperidinecarboxamide

Preferred are compounds (80), (109), (110), (112), (115), (142), (143), (144), (145), (153), (157), (163), (165), (166) and (179).

The compound to be used as the Rho kinase inhibitor of the present invention is, for example, a compound of the formula (II), which is exemplified by the following compounds.

(204) 1-(5-isoquinolinesulfonyl)homopiperazine
(205) 1-(5-isoquinolinesulfonyl)-2-methylhomopiperazine
(206) 1-(5-isoquinolinesulfonyl)-3-methylhomopiperazine
(207) 1-(5-isoquinolinesulfonyl)-6-methylhomopiperazine
(208) 1-(5-isoquinolinesulfonyl)-2,3-dimethylhomopiperazine
(209) 1-(5-isoquinolinesulfonyl)-3,3-dimethylhomopiperazine
(210) 1-(5-isoquinolinesulfonyl)-3-ethylhomopiperazine
(211) 1-(5-isoquinolinesulfonyl)-3-propylhomopiperazine
(212) 1-(5-isoquinolinesulfonyl)-3-isobutylhomopiperazine
(213) 1-(5-isoquinolinesulfonyl)-3-phenylhomopiperazine
(214) 1-(5-isoquinolinesulfonyl)-3-benzylhomopiperazine
(215) 1-(5-isoquinolinesulfonyl)-6-ethylhomopiperazine
(216) 1-(5-isoquinolinesulfonyl)-6-propylhomopiperazine
(217) 1-(5-isoquinolinesulfonyl)-6-butylhomopiperazine
(218) 1-(5-isoquinolinesulfonyl)-6-pentylhomopiperazine
(219) 1-(5-isoquinolinesulfonyl)-6-hexylhomopiperazine
(220) 1-(5-isoquinolinesulfonyl)-6-phenylhomopiperazine
(221) 1-(5-isoquinolinesulfonyl)-6-benzylhomopiperazine

(222) 1-(5-isoquinolinesulfonyl)-4-methylhomopiperazine
(223) 1-(5-isoquinolinesulfonyl)-4-ethylhomopiperazine
(224) 1-(5-isoquinolinesulfonyl)-4-propylhomopiperazine
(225) 1-(5-isoquinolinesulfonyl)-4-butylhomopiperazine
(226) 1-(5-isoquinolinesulfonyl)-4-hexylhomopiperazine
(227) N-(2-aminoethyl)-1-chloro-5-isoquinolinesulfonamide
(228) N-(4-aminoethyl)-1-chloro-5-isoquinolinesulfonamide
(229) N-(2-amino-1-methylethyl)-1-chloro-5-isoquinolinesulfonamide
(230) N-(2-amino-1-methylpentyl)-1-chloro-5-isoquinoline
(231) N-(3-amino-2-methylbutyl)-1-chloro-5-isoquinolinesulfonamide
(232) N-(3-di-n-butylaminopropyl)-1-chloro-5-isoquinolinesulfonamide
(233) N-(N-cyclohexyl-N-methylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(234) N-(2-guanidinoethyl)-1-chloro-5-isoquinolinesulfonamide
(235) N-(2-guanidinobutyl)-1-chloro-5-isoquinolinesulfonamide
(236) N-(2-guanidino-1-methylethyl)-1-chloro-5-isoquinolinesulfonamide
(237) N-(2-guanidinomethylpentyl)-1-chloro-5-isoquinolinesulfonamide
(238) N-(2-guanidino-3-methylbutyl)-1-chloro-5-isoquinolinesulfonamide
(239) N-(3-guanidino-2-methylpropyl)-1-chloro-5-isoquinolinesulfonamide
(240) N-(4-guanidino-3-methylbutyl)-1-chloro-5-isoquinolinesulfonamide
(241) 2-methyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(242) 2-ethyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(243) 2-isobutyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(244) 2,5-dimethyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(245) 1-methyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(246) 1-amidino-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(247) 1-amidino-4-(1-chloro-5-isoquinolinesulfonyl)homopiperazine
(248) 1-amidino-3-methyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(249) 1-amidino-2,5-dimethyl-4-(1-chloro-5-isoquinolinesulfonyl)piperazine
(250) N-(2-aminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(251) N-(4-aminobutyl)-1-hydroxy-5-isoquinolinesulfonamide
(252) N-(2-amino-1-methylethyl)-1-hydroxy-5-isoquinolinesulfonamide
(253) N-(2-amino-1-methylheptyl)-1-hydroxy-5-isoquinolinesulfonamide
(254) N-(3-amino-2-methylbutyl)-1-hydroxy-5-isoquinolinesulfonamide
(255) N-[3-(N, N-dibutylamino)propyl]-1-hydroxy-5-isoquinolinesulfonamide
(256) N-[2-(N-cyclohexyl-N-methylamino)ethyl]-1-hydroxy-5-isoquinolinesulfonamide
(257) N-(2-guanidinoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(258) N-(4-guanidinobutyl)-1-hydroxy-5-isoquinolinesulfonamide
(259) N-(2-guanidino-1-methylethyl)-1-hydroxy-5-isoquinolinesulfonamide
(260) N-(1-guanidinomethylpentyl)-1-hydroxy-5-isoquinolinesulfonamide
(261) N-(2-guanidino-3-methylbutyl)-1-hydroxy-5-isoquinolinesulfonamide
(262) N-(3-guanidino-2-methylpropyl)-1-hydoxy-5-isoquinolinesulfonamide
(263) N-(4-guanidino-3-methylbutyl)-1-hydroxy-5-isoquinolinesulfonamide
(264) 2-methyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(265) 2-ethyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(266) 2-isobutyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(267) 2,5-dimethyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(268) 1-methyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(269) 1-amidino-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(270) 1-amidino-4-(1-hydroxy-5-isoquinolinesulfonyl)homopiperazine
(271) 1-amidino-3-methyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(272) 1-amidino-2,5-dimethyl-4-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(273) N-(2-methylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(274) N-(2-ethylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(275) N-(2-propylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(276) N-(2-butylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(277) N-(2-hexylaminoethyl)-1-chloro-5-isoquinolinesulfonamide
(278) 1-(1-chloro-5-isoquinolinesulfonyl)piperazine
(279) 1-(1-chloro-5-isoquinolinesulfonyl)homopiperazine

(280) N-(2-methylaminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(281) N-(2-ethylaminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(282) N-(2-propylaminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(283) N-(2-butylaminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(284) N-(2-hexylaminoethyl)-1-hydroxy-5-isoquinolinesulfonamide
(285) 1-(1-hydroxy-5-isoquinolinesulfonyl)piperazine
(286) 1-(1-hydroxy-5-isoquinolinesulfonyl)homopiperazine
(287) 1-(5-isoquinolinesulfonyl)-4-methylpiperazine
(288) 1-(5-isoquinolinesulfonyl)-4-n-hexylpiperazine
(289) 1-(5-isoquinolinesulfonyl)-4-cinnamylpiperazine
(290) 1-(5-isoquinolinesulfonyl)piperazine
(291) N-(2-aminoethyl)-5-isoquinolinesulfonamide
(292) N-(4-aminobutyl)-5-isoquinolinesulfonamide
(293) N-(3-di-n-butylaminopropyl)-5-isoquinolinesulfonamide
(294) 1-(5-isoquinolinesulfonyl)-3-methylpiperazine
(295) 1-(5-isoquinolinesulfonyl)-3-isobutylpiperazine
(296) 1-(5-isoquinolinesulfonyl)-2,5-dimethylpiperazine
(297) N-(3-guanidino-2-phenylpropyl)-5-isoquinolinesulfonamide
(298) N-(6-guanidino-1-methylheptyl)-5-isoquinolinesulfonamide
(299) 2-[2-(5-isoquinolinesulfonamide)ethylamino]-2-imidazoline
(300) 2-amidino-1-(5-isoquinolinesulfonyl)piperazine
(301) 4-amidino-2,5-dimethyl-1-(5-isoquinolinesulfonyl)piperazine
(302) 4-amidino-1-(5-isoquinolinesulfonyl)homopiperazine
(303) 4-(N1, N2-dimethylamidino)-1-(5-isoquinolinesulfonyl)piperazine
(304) 4-amidino-3-butyl-1-(5-isoquinolinesulfonyl)piperazine
(305) 4-hexyl-1-(5-isoquinolinesulfonyl)ethylenediamine
(306) N-(4-guanidinobutyl)-5-isoquinolinesulfonamide
(307) N-(2-guanidinoethyl)-5-isoquinolinesulfonamide
(308) 1-(5-isoquinolinesulfonyl)-2-methylpiperazine

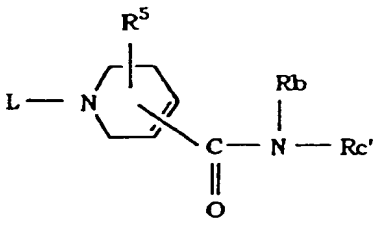
[0101] Preferred are compounds (204) and (308).

[0102] The compound to be used as the Rho kinase inhibitor of the present invention may be a pharmaceutically acceptable acid addition salt. The acid is exemplified by inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid and the like and organic acid such as methanesulfonic acid, fumaric acid, maleic acid, mandelic acid, citric acid, tartaric acid, salicylic acid and the like. The compound having a carboxyl group can be converted to a salt with a metal such as sodium, potassium, calcium, magnesium, aluminum and the like or a salt with amino acid such as lysine and the like. In addition, their monohydrate, dihydrates, 1/2 hydrates, 1/3 hydrates, 1/4 hydrates, 2/3 hydrates, 3/2 hydrates and the like are also encompassed in the present invention.

[0103] The compound of the formula (I) can be synthesized according to the method disclosed in Japanese Patent Unexamined Publication No. 62-89679, Japanese Patent Unexamined Publication No. 3-218356, Japanese Patent Unexamined Publication No. 5-194401, Japanese Patent Unexamined Publication No. 6-41080, WO95/28387 and the like.

[0104] The compound of the formula (II) can be synthesized according to the method disclosed in Japanese Patent Unexamined Publication No. 57-156463, Japanese Patent Unexamined Publication No. 57-200366, Japanese Patent Unexamined Publication No. 58-121278, Japanese Patent Unexamined Publication No. 58-121279, Japanese Patent Unexamined Publication No. 59-93054, Japanese Patent Unexamined Publication No. 60-81168, Japanese Patent Unexamined Publication No. 61-152658, Japanese Patent Unexamined Publication No. 61-227581, Japanese Patent Unexamined Publication No. 62-103066, USP-4678783 and the like.

[0105] Of the compounds of the formula (I), a compound wherein Ra is a group of the formula (c) and Rc is Rc', namely, an amide compound of the formula (III)



[III]

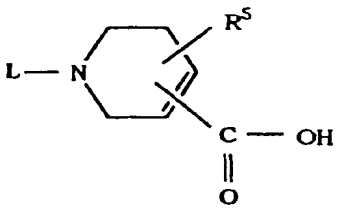
wherein Rc' is an optionally substituted heterocycle containing nitrogen of the above-mentioned Rc except pyridine, and other symbols are as defined above, is a novel compound which can be synthesized by the following methods.

Method 1

[0106] A compound of the formula (IV)

$$Rc'\text{-}NH\text{-}Rb \qquad (IV)$$

wherein each symbol is as defined above, and a compound of the formula (V)



(V)

wherein each symbol is as defined above, or a reactive derivative thereof are reacted to give the compound. The reactive derivative of carboxylic acid compound is exemplified by acid halide, ester, acid anhydride, mixed acid anhydride and the like.

[0107] This reaction beneficially proceeds by stirring in the presence of a solvent inert to the reaction, such as tetrahydrofuran, dioxane, chloroform, dichloromethane, dimethylformamide, benzene, toluene, ethanol and the like. Water, alcohol or acid liberated during the reaction is removed from the reaction mixture by a method known in the pertinent field, such as azeotropic distillation, forming a complex, converting to salt and the like.

Method 2

[0108] Of the compounds of the formula (III), a compound wherein L has a substituent other than hydrogen can be produced by reacting a compound wherein L is hydrogen, with a compound of the formula (VI)

$$L^1\text{-}M \qquad (VI)$$

wherein L^1 is, of the aforementioned L, a substituent other than hydrogen and M is a reactive atom, according to N-alkylation or N-acylation known in this field.

Method 3

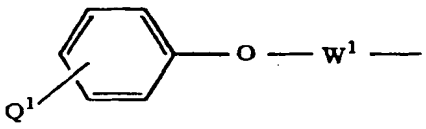
[0109] Of the compounds of the formula (III), a compound wherein L is alkyl or has a substituent having the formula (i) can be produced by reductive amination reaction of a compound wherein L is hydrogen and a compound of the formula (VII)

$$L^2\text{=}C\text{=}O \qquad (VII)$$

wherein L^2 is a group that can be converted to alkyl or a group of the formula(i), by reductive amination reaction.

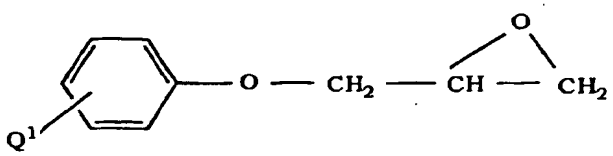
Method 4

[0110] Of the compounds of the formula (III), a compound wherein L is a group of the formula (I)



(I)

wherein Q^1 is as defined above and W^1 is hydroxytrimethylene from among the substituents at W, can be produced by reacting a compound of the formula (III) wherein L is hydrogen and a compound of the formula (VIII)



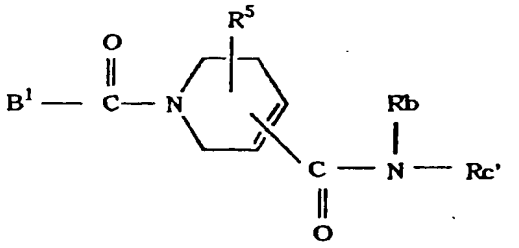
(VIII)

wherein Q^1 is as defined above.

[0111] The reaction advantageously proceeds in a suitable solvent which does not influence the reaction, such as alcohol (e.g., methanol, ethanol, 2-propanol and the like), aliphatic or alicydlic ketone (e.g., 2-propanone, 2-butanone, cyclohexane and the like) and the like. Addition of a suitable base such as alkali metal carbonate, hydrogencarbonate and the like enables acceleration of the reaction rate. The reaction temperature is rather elevating, which is preferably refluxing temperature of the reaction mixture.

Method 5

[0112] Of the compounds of the formula (III), a compound wherein L is hydrogen can be produced from a compound of the formula (III-a)



(III-a)

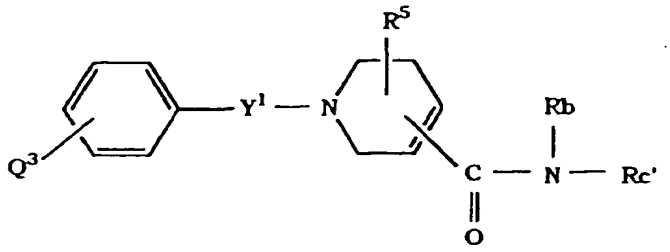
wherein B^1 is alkoxy or aralkyloxy, from among the aforementioned substituents B, and other symbols are as defined above.

[0113] Of the compounds (III-a), a compound wherein B^1 is alkoxy is stirred in a suitable organic solvent which does not influence the reaction, such as alcohol (e.g., methanol, ethanol, 2-propanol and the like) and ether (e.g., tetrahydrofuran and the like) in the presence of a suitable base, such as hydroxide of alkali metal or alkaline earth metal, carbonate or hydrogencarbonate (e.g., sodium hydroxide, potassium carbonate, sodium hydrogencarbonate and the like) and heated as necessary to give a compound of the formula (III) wherein L is hydrogen.

[0114] Of the compounds (III-a), a compound wherein B^1 is aralkyloxy is subjected to reductive decomposition reaction in a suitable organic solvent which does not influence the reaction in the presence of a suitable catalyst such as

palladium carbon and the like using a hydrogen source of hydrogen, hydrazine, formic acid, ammonium formate and the like at normal temperature or under pressurization where necessary.

[0115] Moreover, a compound (III-a) is stirred in 5-35%, preferably 15-30%, acetic acid in the presence of hydrogen bromide, whereby the compound can be converted. A compound of the formula (III-b)



(III-b)

wherein Y^1 is methylene, from among the aforementioned substituents Y, and other symbols are as defined above, is subjected to catalytic hydrogenation decomposition reaction wherein the compound is stirred in a suitable organic solvent which does not influence the reaction in the presence of a suitable catalyst such as palladium carbon and the like under hydrogen to give a compound of the formula (III) wherein L is hydrogen.

[0116] The compound of the formula (III) thus obtained can be separated from the reaction mixture and purified by a method known in the field of art, such as recrystallization, chromatography and the like.

[0117] In addition, the compound of the formula (III) can form a pharmaceutically acceptable salt by a conventional method. The acid to be used for forming a salt can be appropriately selected from inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid and the like, organic acids such as methanesulfonic acid, fumaric acid, maleic acid, mandelic acid, citric acid, tartaric acid, salicylic acid and the like, amino acids such as lysine and the like, and metal such as sodium, potassium, calcium, magnesium, aluminum and the like. These acid addition salts can be converted to a corresponding free base by the reaction with alkali such as sodium hydroxide, potassium hydroxide and the like according to a known method. The salts can be also converted to quaternary ammonium.

[0118] The compound of the formula (III) may exist as optical isomer, racemate thereof or cis-trans isomer, all of which are encompassed in the present invention. These isomers can be isolated by a conventional method or produced by using various starting compounds.

[0119] When the Rho kinase inhibitor of the present invention is used as a pharmaceutical agent, particularly as a therapeutic agent of hypertension, a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a prophylactic agent of immature birth, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, a contraceptive, a prophylactic agent of digestive tract infection, an anti-AIDS drug, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy or a brain function improving drug, it can be prepared as a general pharmaceutical agent. For example, the Rho kinase inhibitor of the present invention is mixed with a pharmaceutically acceptable carrier (e.g., excipient, binder, disintegrator, corrective, corrigent, emulsifier, diluent, solubilizer and the like) to give a pharmaceutical composition or a pharmaceutical preparation in the form of tablet, pill, powder, granule, capsule, troche, syrup, liquid, emulsion, suspension, injection (e.g., liquid, suspension and the like), suppository, inhalant, percutaneous absorber, eye drop, eye ointment and the like in the form suitable for oral or parenteral preparation.

[0120] When preparing a solid preparation, an additive such as sucrose, lactose, cellulose sugar, D-mannitol, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectines, tragacanth, gum arabic, gelatins, collagens, casein, albumin, calcium phosphate, sorbitol, glycine, carboxymethyl cellulose, polyvinylpyrrolidone, hydroxypropylcellulose, hydroxypropylmethylcellulose, glycerol, polyethyleneglycol, sodium hydrogencarbonate, magnesium stearate, talc and the like are used. Tablets can be applied with a typical coating, where necessary, to give sugar coated tablets, enteric tablets, film-coated tablets, two-layer tablets and multi-layer tablets.

[0121] When preparing a semi-solid preparation, animal and plant fats and oils (e.g., olive oil, corn oil, castor oil and the like), mineral fats and oils (e.g., petrolatum, white petrolatum, solid paraffin and the like), wax (e.g., jojoba oil, carnauba wax, bee wax and the like), partly or entirely synthesized glycerol fatty acid esters (e.g., lauric acid, myristic acid, palmitic acid and the like), and the like are used. Examples of commercially available products of these include Witepsol (manufactured by Dynamitnovel Ltd.), Farmazol (NOF Corporation) and the like.

[0122] When preparing a liquid preparation, an additive, such as sodium chloride, glucose, sorbitol, glycerol, olive oil, propylene glycol, ethyl alcohol and the like, is used. In particular, when preparing an injection, a sterile aqueous solution such as physiological saline, isotonizing liquid, oily liquid (e.g., sesame oil and soybean oil) and the like is used. Where

necessary, a suitable suspending agent such as sodium carboxymethylcellulose, nonionic surfactant, solubilizer (e.g., benzyl benzoate and benzyl alcohol), and the like can be concurrently used. Moreover, when an eye drop is prepared, an aqueous liquid or solution is used, which is particularly a sterile injectable aqueous solution. The liquid for an eye drop can appropriately contain various additives such as buffer (preferred are borate buffer, acetate buffer, carbonate buffer and the like for less irritation), isotonizing agent, solubilizer, preservative, thickener, chelating agent, pH adjuster (preferably, pH is generally adjusted to about 6-8.5) and aromatic.

[0123] The content of the active ingredient in these preparation is 0.1-100 wt%, suitably 1-50 wt%, of the preparation. While subject to variation depending on the condition, body weight, age and the like of patient, in general, about 1-500 mg of the active ingredient is orally administered daily for an adult in a single dose or several doses.

Examples

[0124] The present invention is described in more detail in the following by way of Examples, Formulation Examples and pharmacological action, to which the present invention is not limited.

[0125] In the following, the synthetic method of the novel compound of the formula (III) of the present invention is described by referring to examples.

Example 1

[0126]

(a) N-Benzyloxycarbonylisonipecotyl chloride (5 g) was added to a solution of 4-amino-1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridine (3 g) and diisopropylethyamine (2.16 g) in acetonitrile (40 ml) and the mixture was stirred at room temperature for 2 hours. The reaction mixture was poured into ice-water and extracted with chloroform. The residue obtained by water washing, drying and then concentration under reduced pressure was purified by silica gel column chromatography to give 6.3 g of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyloxycarbonyl-4-piperidinecarboxamide.

PMR($CDCl_3$) : 1.67(9H, s), 1.79(2H, m), 1.95(2H, m), 2.53(1H, m), 2.89(2H, m), 4.29(2H, m), 5.15(2H, s), 6.48(1H, d,J=4.4Hz), 7.36(5H, m), 7.59(1H, br), 7.61(1H, d,J=4.4Hz), 7.99(1H, d,J=5.4Hz), 8.43(1H, d,J=5.4Hz)

(b) N-(1-tert-Butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyloxycarbonyl-4-piperidinecarboxamide (2 g) was dissolved in methanol (30 ml) and 100% palladium carbon hydroxide (0.5 g) was added for hydrogenation (normal pressure). After the completion of the reaction, the catalyst was filtered off and the filtrate was concentrated under reduced pressure to give 1.2 g of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide.

PMR(DMSO-d_6) : 1.59(9H, s), 1.83(2H, m), 2.01(2H, m), 2.89(2H, m), 3.01(1H, m), 3.32 (2H, m), 7.19(1H, d,J=4.4Hz), 7.68(1H, d,J=4.4Hz), 7.97(1H, d,J=5.4Hz), 8.24(1H, d,J=5.4Hz), 8.81(1H, br), 10.45(1H, s)

(c) Formic acid (10 ml) was added to N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide (1 g) and the mixture was stirred at room temperature for 2 hours. The mixture was neutralized with aqueous 1N sodium hydroxide solution and extracted with chloroform. The crystals obtained by water washing, drying and then concentration under reduced pressure were dissolved in 15% hydrochloric acid-methanol solution (5 ml). The crystals obtained by concentration of the resulting solution were recrystallized from ethanol-ethyl acetate to give 650 mg of N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide mono hydrochloride monohydrate, melting point 273°C (decomposition).

PMR(DMSO-d_6) : 1.52(2H, m), 1.69(2H, m), 2.51(2H, m), 2.70(1H, m), 2.97(2H, m), 3.32(1H, br), 6.79(1H, d,J=3.4Hz), 7.31(1H, d,J=3.4Hz), 7.79(1H, d,J=5.4Hz), 8.04(1H, d,J=5.4Hz), 9.82(1H, s), 11.54(1H, br)

Example 2

[0127]

(a) A solution of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide (0.6 g), phenetyl bromide (390 mg) and potassium carbonate (290 mg) in dimethylformamide (10 ml) was stirred at 80°C for 2 hours. The reaction mixture was poured into ice water and extracted with chloroform. The residue obtained by water washing, drying and then concentration under reduced pressure was purified by silica gel column chromatography to give 550 mg of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide.

PMR(DMSO-d_6) : 1.59(9H, s), 1.66(2H, m), 1.80(2H, m), 1.98(2H, m), 2.50(2H, m), 2.56(1H, m), 2.74(2H, m), 3.01(2H, m), 7.05(1H, d,J=4.4Hz), 7.23(5H, m), 7.68(1H, d,J=4.4Hz), 7.97(1H,J=5.4Hz), 8.23(1H, d,J=5.4Hz), 10.03(1H, s)

(b) Formic acid (5 ml) was added to N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide (550 mg) and the mixture was stirred at room temperature for 2 hours. The mixture was neutralized with aqueous 1N sodium hydroxide solution and extracted with chloroform. The crystals obtained by water washing, drying and then concentration under reduced pressure were dissolved in 15% hydrochloric acid-methanol solution (1 ml). The crystals obtained by concentration of the resulting solution were recrystallized from ethanol-ethyl acetate to give 250 mg of N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide dihydrochloride 1/4 hydrate, melting point 272°C (decomposition).
PMR(DMSO-d_6/TMS) : 2.00-2.19(4H, m), 2.93-3.41(7H, m), 3.63-3.68(2H, m), 7.22-7.37(5H, m), 7.50(1H, d,J=2.0Hz), 7.56(1H, t,J=2.0Hz), 8.25(1H, d,J= 6.8Hz), 8.33(1H, d,J=6.8Hz), 10.86(1H, br), 11.36(1H, s), 12.77(1H, br)

Example 3

[0128]

(a) A solution of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide (500 mg), benzyl bromide (370 mg) and potassium carbonate (300 mg) in dimethylformamide (10 ml) was stirred at 80°C for 4 hours. The reaction mixture was poured into ice-water and extracted with chloroform. The residue obtained by water washing, drying and then concentration under reduced pressure was purified by silica gel column chromatography to give 300 mg of N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyl-4-piperidinecarboxamide.
PMR($CDCl_3$) : 1.65(9H, s), 1.91(4H, m), 2.04(2H, m), 2.35(1H, m), 2.97(2H, m), 3.51(2H, s), 6.44(1H, d,J=3.9Hz), 7.30(5H, m), 7.49(1H, br), 7.57(1H, d,J=3.9Hz), 7.99(1H, d,J=5.4Hz), 8.41(1H, d,J=5.4Hz)
(b) Formic acid (4 ml) was added to N-(1-tert-butoxycarbonyl-1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyl-4-piperidinecarboxamide (300 mg) and the mixture was stirred at room temperature for 1 hour. The mixture was neutralized with aqueous 1N sodium hydroxide solution and extracted with chloroform. The crystals obtained by water washing, drying and then concentration under reduced pressure were dissolved in 15% hydrochloric add-methanol solution (1 ml). The crystals obtained by concentration of the resulting solution were recrystallized from ethanol-ethyl acetate to give 120 mg of N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-benzyl-4-piperidinecarboxamide dihydrochloride monohydrate, melting point 260°C (decomposition).
PMR(DMSO-d_6/TMS) : 2.00-2.15(4H, m), 2.92-2.98(2H, m), 3.13-3.19(1H, m), 3.36-3.43(2H, m), 4.32(2H, s), 7.55(1H, br), 7.63(2H, m), 8.20(1H, d,J=6.4Hz), 8.31(1H, d,J=6.4Hz), 10.76(1H, br), 11.25(1H, br), 12.69(1H, br)

[0129] The following compounds can be obtained in the same manner as in the above Examples.

Example 4

N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-4-piperidinecarboxamide dihydrochloride 3/2 hydrate, melting point 277°C (decomposition)

Example 5

N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-aminoacetyl-4-piperidinecarboxamide dihydrochloride 1/2 hydrate, melting point 264°C (decomposition)

Example 6

N-(1-methoxymethyl-1H-pyrazolo[3,4-b]pyridin-4-yl)-4-piperidinecarboxamide monohydrate, melting point 240-241°C

Example 7

N-(2,3-dihydro-1H-pyrrolo[2,3-b]pyridin-4-yl)-4-piperidinecarboxamide dihydrochloride 3/2 hydrate, melting point 235°C (decomposition)

Example 8

N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-amidino-4-piperidinecarboxamide dihydrochloride 5/4 hydrate, melting point 246°C (decomposition)

Example 9

N-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1-(3-phenylpropyl)-4-piperidinecarboxamide dihydrochloride, melting point 276°C (decomposition)

Example 10

N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-(2-phenylethyl)-4-piperidinecarboxamide dihydrochloride hydrate, melting point 259-261°C (decomposition)

Example 11

N-(1H-pyrazolo[3,4-b]pyridin-4-yl)-1-(3-phenylpropyl)-4-piperidinecarboxamide dihydrochloride 1/2 hydrate, melting point 240-244°C (decomposition)

[0130] A method for preparing the pharmaceutical preparation of the present invention is explained in the following.

Formulation Example 1 : tablets

[0131]

Inventive compound	10.0 mg
Lactose	50.0 mg
Corn starch	20.0 mg
Crystalline cellulose	29.7 mg
Polyvinylpyrrolidone K30	5.0 mg
Talc	5.0mg
Magnesium stearate	0.3mg

(continued)

	120.0 mg

[0132] The inventive compound, lactose, corn starch and crystalline cellulose were mixed, kneaded with polyvinylpyrrolidone K30 paste solution and passed through a 20-mesh sieve for granulation. After drying at 50°C for 2 hours, the granules were passed through a 24-mesh sieve, and talc and magnesium stearate were added. Using a Ø7 mm punch, tablets weighing 120 mg per tablet were prepared.

Formulation Example 2 : Capsules

[0133]

Inventive compound	10.0 mg
Lactose	70.0 mg
Corn starch	35.0 mg
Polyvinylpyrrolidone K30	2.0 mg
Talc	2.7mg
Magnesium stearate	0.3mg
	120.0 mg

[0134] The inventive compound, lactose, corn starch and crystalline cellulose were mixed, kneaded with polyvinylpyrrolidone K30 paste solution and passed through a 20-mesh sieve for granulation. After drying at 50°C for 2 hours, the granules were passed through a 24-mesh sieve and talc and magnesium stearate were added. The mixture was filled in hard capsules (No. 4) to give capsules weighing 120 mg.

[0135] The pharmacological action of the pharmaceutical preparation of the present invention is explained in the following by way of experimental examples.

Experimental Example 1 : Rho kinase inhibitory action (inhibition of bovine aorta thoracia Rho kinase)

[0136] The Rho kinase was prepared from bovine aorta of thorax by partial purification as in the following. The artery was minced and homogenized with a 9-fold amount of 50 mM Tris-hydroxymethylaminomethane (Tris) (pH=7.4), 1 mM dithiothreitol, 1 mM EGTA, 1 mM EDTA, 100 μM p-amidinophenylmethylsulfonyl fluoride, 5 μM E-64, 5 μM leupeptine and 5 μM pepstatin A. The homogenate was centrifuged (10,000 × g, 30 minutes) to give supernatant. The supernatant was adsorbed onto a hydroxyapatite column. The column was washed with 0.2M phosphate buffer (pH=6.8). The standard product of Rho kinase was eluted with 0.4M phosphate buffer (pH=6.8). The Rho kinase was assayed as follows.

[0137] A reaction mixture (total amount 50 μl) containing 50 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, 50 μg/ml histone, 10 μM GTPγS, 100 μg/ml Rho, 2 μM [^{32}P]ATP, the Rho kinase (3 μl) prepared in the above and the test compound was reacted at 30°C for 5 minutes. The reaction was terminated by the addition of 25% trichloroacetic acid (TCA) solution (1 ml) and the mixture was stood at 4°C for 30 minutes. Then, the mixture was filtered through a membrane filter (HAWP type, Millipore), and the radioactivity of the filter was counted on a liquid scintillation counter. The inhibitory action of the test compound was calculated from the following formula based on the comparison of the radioactivity with the sample without the test compound (control). The results are shown in Table 1.

$$\text{Inhibition (\%)} = \left[\frac{\text{cpm under control} - \text{cpm in the presence of test compound}}{\text{cpm under control}}\right] \times 100$$

Table 1

Test compound		Inhibition (%)
Compound 109.2HCl	(1 μM)	81
	(10 μM)	100
Compound 165.2HCl.3/2H_2O (10 μM)		100
Compound 80.2HCl.H_2O (10 μM)		100
Compound 204.2HCl (10 μM)		93

Experimental Example 2 : Rho kinase inhibitory action (inhibition of human platelet Rho kinase (p160ROCK))

[0138] Human platelet p160ROCK was isolated by the method of Ishizaki et al. (Ishizaki T et al., The EMBO J., 15(8), 1885-1893, 1996).

[0139] Kinase assay included the following steps. That is, a reaction mixture (total amount 30 μl) containing 50 mM Hepes-NaOH (pH=7.4), 10 mM $MgCl_2$,5 mM $MnCl_2$, 2 mM dithiothreitol, 0.02% Brij35, 1 μM [γ-^{32}P]ATP, 330 μg/ml histone, p160ROCK (2 μl) isolated by the method of Ishizaki et al. and the test compound was incubated at 30°C for 20 minutes. The solution was mixed with a 1/3 amount of 4 × Laemmli sample buffer, boiled for 5 minutes and applied to SDS-PAGE. The gel was stained with Coomassie Brilliant Blue and dried. The band of histone was cut out and assayed for radioactivity. The test compound was evaluated in the same manner as in Experimental Example 1, and the concentration of each test compound necessary for 50% inhibition was calculated as IC50 (μM). The results are shown in Table 2.

Table 2

Test compound	IC_{50} (μM)
Compound 80.2HCl.H_2O	1.5
Compound 109.2HCl	0.11
Compound 143.2HCl.H_2O	1.6
Compound 204.2HCl	3.8
Compound 308.2HCl	5.0

Experimental Example 3 : Rho kinase inhibitory action (inhibition of p160ROCK and ROCKII)

[0140] The standard enzyme products of p160ROCK (Ishizaki T et al., The EMBO J., 15(8), 1885-1893, 1996) and ROCKII (Nakagawa O et al., FEBS Lett. 392 189-193, 1996) were obtained in the following manner. COS cells were seeded in a 3.5 cm dish and incubated overnight. Using lipofectamine, the expression vectors of p160ROCK and ROCKII (pCAG-myc-p160ROCK and pCAG-myc-ROCKII: see Ishizaki T et al., The EMBO J., 15(8), 1885-1893, 1996 and Nakagawa O et al., FEBS Lett. 392 189-193, 1996) were transfected. After incubation for 20 hours, the cells were washed once with ice-cooled PBS, and the cells were lysed on ice for 20 minutes using a lysis buffer (20 mM Tris-HCl (pH=7.5), 1 mM EDTA, 1 mM EGTA, 5 mM $MgCl_2$, 25 mM NaF, 10 mM β glycerophosphate, 5 mM sodium pyrophorphate, 0.2 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, 0.2 mM sodium vanadate, 0.05%Triton X-100, 0.1 μM calyculin A). The lysate was centrifuged at 10,000 × g for 10 minutes and the supernatant was recovered. To the supernatant was added 9E10 anti-myc epitope antibody (see Ishizaki T et al., The EMBO J., 15(8), 1885-1893, 1996) and the mixture was shaken for 2 hours. Then, protein G-Sepharose was added and the mixture was shaken for 2 more hours. The suspension was centrifuged at 1,000 × g for 5 minutes and the resulting pellets were washed 3 times with lysis buffer and once with kinase buffer (50 mM Hepes-NaOH (pH=7.4), 10 mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM dithiothreitol, 0.02% Brij35). The pellets were suspended in kinase buffer to give a standard enzyme product. The kinase assay followed the method shown in Experimental Example 2, wherein the standard enzyme product obtained in this Experimental Example was used instead of human platelet Rho kinase (p160ROCK). The concentration of each test compound necessary for 50% inhibition was calculated as IC50 (μM). The results are shown in Table 3.

Table 3

Test compound	IC_{50} (μM)	
	p160ROCK	ROCK-II
Compound 80.2HCl.H_2O	0.63	0.56
Compound 109.2HCl	0.095	0.048
Compound 143.2HCl.H_2O	0.88	0.47
Compound 204.2HCl	2.3	1.1

Experimental Example 4 : vasodilating action

[0141] Male rabbits (body weight 1.9-3.0 kg) were anesthetized with pentobarbital sodium and exsanguinated, whereafter thoractic aorta was removed. An about 2 mm width aortic ring samples were prepared and hung in a Magnus bath (40 ml) filled with Krebs-Henseleit solution (37°C, NaCl 117 mM ; KCl 4.7 mM ; $CaCl_2$ 2.5 mM; $MgSO_4$ 1.2mM ; $NaHCO_3$ 24.8mM ; KH_2PO_4 1.2mM ; glucose 11.0 mM) at a load of 2 g. The Magnus bath was constantly bubbled with a mixed gas (95% O_2+5% CO_2 gas). The tension of the preparation was measured with an isomeric transducer (TB-611T, Nippon Koden). The preparation was contracted with phenylephrine (10^{-6} M) and, after the contraction was stabilized, the test compound was added accumulatively and relaxing action was observed. The relaxing action of the test compound was calculated by expressing the concentration of the test compound necessary for 50% relaxation as IC50 (μM) against the contraction with phenylephrine as 100%. The results are shown in Table 4.

Experimental Example 5 : Effect on contraction by acetylcholine of trachea specimen removed from guinea pig

[0142] Male Hartley guinea pigs (body weight 260-390 g) were anesthetized by the peritoneal administration of pentobarbital sodium (100 mg/kg) and exsanguinated, whereafter trachea was removed. The anterior cartilage of the trachea was opened and the band was cut in a 3 mm width strip to give a specimen. The specimen was hung in a Magnus bath (40 ml) filled with Krebs-Henseleit solution (NaCl 117 mM ; KCl 4.7 mM ; $CaCl_2$ 2.5 mM ; $MgSO_4$ 1.2 mM ; $NaHCO_3$ 24.8 mM ; KH_2PO_4 1.2 mM ; glucose 11.0 mM) at a load of 1 g. The Magnus bath was constantly bubbled with a mixed gas (95% O_2+5% CO_2 gas). The tension of the strip was measured with an isomeric transducer (TB-611T, Nippon Koden) and depicted on a recorder (Ti-102, Tokai Irika). The strip was contracted with acetylcholine (10^{-6} M) and, after the contraction was stabilized, the test compound was added accumulatively and relaxing reaction was observed. The relaxing action of the test compound was calculated and expressed by the concentration of the test compound necessary for 50% relaxation as IC50 (μM) against the maximum response with papaverine (10^{-4} M) as 100%. The results are shown in Table 4.

Table 4

Test compound	Vasorelaxing action (μM)	Trachea relaxing action (μM)
Compound 80.2HCl.H_2O	0.70	0.56
Compound 109.2HCl	0.1	0.043
Compound 165.2HCl.3/2H_2O	0.051	0.066
Compound 179.2HBr.1/2H_2O	0.03	0.029

Experimental Example 6 : Peripheral blood flow increasing action

[0143] Streptozotocin (STZ, 65 mg/kg) was intravenously injected to male SD rats (body weight 200-300 g) to prepare diabetic rats. One month later, STZ-induced diabetic rats were anesthetized with pentobarbital sodium and the blood flow in the hind limb skin was measured with laser blood flowmeter (ALF21R, Advance). The test compound was intravenously administered via catheter dwelled in the carotid arteries, and hind limb skin blood flow increasing action was

observed. The blood flow increasing action of the test compound was expressed by increase percentage from the blood flow before administration. The results are shown in Table 5.

Table 5

Test compound		Increase in skin blood flow± standard error (%)
Compound 80.2HCl.H_2O (1 μg)		135.0±13.4
Compound 157.HCl.H_2O (1 μg)		211.6 ±13.6
Compound 165.2HCl.3/2H_2O	(0.03 μg)	135.8± 0.0
	(0.1 μg)	144.7± 0.0
Compound 166.2HCl.H_2O	(0.3 μg)	143.2±25.4
	(1 μg)	165.9±42.5

Experimental Example 7: Inhibition of VLA (very late antigen) integrin activation

[0144] As the index of the activation by VLA integrin, phorbol ester-induced adhesion of CEM cells (humanT cell type established cell) to fibronectin, which is a ligand of VLA integrin, was measured. The inhibitory action on the induced adhesion by the test compound was determined by the following method.

[0145] CEM cells were washed with RPMI1640 medium containing 0.5% bovine serum albumin (BSA), 10 mM HEPES, 2 mM L-glutamin, 1 mM sodium pyruvate, 60 μg/ml kanamycin sulfate and 1.5 mg/ml sodium hydrogencarbonate (hereinafter this medium is referred to as culture solution) and suspended in this medium for use in the following experiment. To each well of a 96 well plate coated with human fibronectin were added GEM cells (5×10^4) and the test compound dissolved in the culture solution (final concentration 1-100 μM) to the amount of 100 μl, and the plate was stood at 37°C for 1 hour. Then, PMA (phorbol 12-myristate 13-acetate, TPA; final concentration 10 ng/ml) and the test compound were added to the amount of 200 μl, and the plate was stood at 37°C for 30 minutes. Each well was washed twice with the culture solution (200 μl) at 37°C, and the LDH (lactate dehydrogenase) activity of the cells adhered to the plate was determined, whereby the amount of the adhered cell was measured. Based on the results obtained by the above-mentioned method, the inhibitory action of the test compound on the induced adhesion was calculated by the following formula The results are shown in Table 6.

$$\text{Inhibition (\%) of adhesion induction} = (a-b)/(a-c) \times 100$$

a= number of cells adhered with the addition of PMA
b= number of cells adhered with the addition of test compound and PMA
c= number of cells adhered without stimulation

Table 6

Test compound	Concentration (μM)	Adhesion induction inhibition (%)
Compound 80.2HCl.H_2O	100	70
Compound 109.2HCl	100	67
Compound 143.2HCl.H_2O	100	77
Compound 165.2HCl.3/2H_2O	10	40
Compound 204.2HCl	100	82
Anti-β1 antibody	20 μg/ml	118
IgG1	20 μg/ml	-25

Experimental Example 8 : Inhibition of bone resorption (in vitro)

[0146] The determination of the in vitro inhibition of bone resorption using mouse femoral bone followed the method below.

[0147] The femoral bone of 3-6 week old male ICR mice was aseptically removed, and bone marrow cavity was washed with F12 medium, containing 10% heat inactivated fetal bovine serum, penicillin G calcium (100 units/ml), kanamycin sulfate (60 μg) and 0.15% sodium hydrogencarbonate (hereinafter the medium is to be referred to as culture solution). After washing the bone marrow cavity and then removing the soft tissue adhered to the bone, the bone was subjected to incubation. The test compound was once dissolved in dimethyl sulfoxide (DMSO) to give a 10 mg/ml solution, which was diluted 1000-fold with the culture solution to give a 10 μg/ml solution. The test compounds were respectively added to the concentration shown in Table 7 and, using this culture solution (1.2 ml), the ICR mouse femoral bone was incubated in a 24 well plate for 6 days under the conditions of 5% CO_2 gas, 95% air. After the completion of the incubation, the culture supernatant was recovered, and the amount of calcium suspending in the culture supernatant was quantitatively determined by the chelate method using o-cresolphthalein. The bone resorption inhibitory action of the test compound was calculated by the following formula using the incubation of the femoral bone without addition of the test compound as a control.

$$\text{Inhibition of bone resorption (\%)} = \left(\frac{\text{Amount of free Ca without addition of test compound} - \text{Amount of free Ca with addition of test compound}}{\text{Amount of free Ca without addition of test compound} - \text{Amount of Ca in culture}} \right) \times 100$$

This experiment was done with 4 cases in each group. As the control, the same amount of DMSO alone as in the case with the addition of the test compound was used. The results are shown in Table 7.

Experimental Example 9 : Inhibition of mouse allogenic mixed lymphocyte reaction

[0148] A mouse allogenic mixed lymphocyte reaction (hereinafter to be referred to as mouse allogenic MLR) was performed by mixed culture (equal ratio) of the spleen cell of BALB/c mice as the reaction cell and the spleen cell of C57BL/6 mice treated with mitomycin C as stimulated cell.

[0149] The reaction cells were prepared by the following method. Spleen was removed from 5-6 week old BALB/c mice and treated with RPMI1640 medium (containing kanamycin sulfate (60 μg/ml), penicillin G potassium (100 units/ml), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate (10 mM), 0.1% sodium hydrogencarbonate and L-glutamin (2 mM)) supplemented with 5% heat inactivated fetal bovine serum (FBS) to give a single cell suspension of the spleen cell. After hemolysis treatment, the suspension was adjusted to 10^7 cells/ml with RPMI1640 medium containing 10^{-4} M 2-mercaptoethanol and 10% FBS and used as a reaction cell suspension.

[0150] The reaction cell suspension (50 μl) prepared by the above method, stimulated cell suspension (50 μl) and the test compound (100 μl) prepared using RPMI1640 medium containing 10% FBS were added to a 96 well plate and incubated at 37°C under 5% CO_2 gas, 95% air for 4 days.

[0151] A pigment assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was applied for the determination of lymphocyte transformation reaction.

[0152] After the completion of culture, the supernatant (100 μl) in each well was removed, and 5 mg/ml MTT solution (20 μl) was added to each well, which was followed by incubation at 37°C for 4 hours. Then, a 0.01 N hydrochloric acid solution (100 μl) containing 10% sodium dodecyl sulfate was added and the mixture was incubated at 37°C overnight. The resulting purple crystals of formazan was dissolved and absorbance at 550 nm was measured using a microplate absorption meter, which was used as the index of lymphocyte transformation reaction of the mouse allogenic MLR. The inhibition of mouse allogenic MLR was evaluated by calculating the inhibition percentage by the following formula. The results are shown in Table 7.

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of MLR with addition of test compound} - \text{absorbance of reacted cells alone}}{\text{Absorbance of MLR without addition of test compound} - \text{absorbance of reacted cells alone}} \right) \times 100$$

Table 7

Test compound	Bone resorption inhibition % (μM)	Mouse allogenic MLR inhibitory activity IC_{50} (μM)
Compound 80.2HCl.H_2O	40.9(100)	9.6
Compound 109.2HCl	42.6(100)	1.6
Compound 112.2HCl	75.7(100)	4.4
Compound 110.2HCl.H_2O	74.0(100)	1.1
Compound 142.2HCl.H_2O	44.2(100)	
Compound 143.2HCl.H_2O	39.4(100)	
Compound 308.2HCl		13.9

Experimental Example 10 : Inhibition of cell growth of SK-Mel-28 melanoma

[0153] Human SK-Mel-28 melanoma (10^4 cells) and the test compound were suspended in RPMI1640 medium containing 100 μl of 10% FBS and incubated in a 96 well plate at 37°C under 5% CO_2 gas for 72 hours. After the incubation, 10 μl of MTT (5 mg/ml) was added to each well and the cells were incubated at 37°C under 5% CO_2 gas for 4 hours. Then, 10% sodium dodecyl sulfate and 0.01 N hydrochloric acid solution were each added by 10 μl to respective wells. After the plate was stood overnight, absorbance at 570 nm was measured using a microplate reader and the inhibition percentage (% cytotoxicity) was calculated by the following formula The results are shown in Table 8.

[0154] The cytotoxicity against human cultured tumor cells was confirmed by pigment method (Carmichael et al., Cancer Res., 47, 936-942, 1987 : Mosman, J. Immunol. Methods, 65, 55-63, 1983) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

[0155] The test compound was dissolved in dimethyl sulfoxide and diluted with RPMI1640 medium before use. The final dimethyl sulfoxide concentration was adjusted to not more than 0.25%.

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance when test compound was added}}{\text{Absorbance when test compound was not added}}\right) \times 100$$

Table 8

Test compound	Cell growth inhibition IC_{50} (μM)
Compound 115.2HBr.1/4H_2O	9
Compound 109.2HCl	58
Compound 142.2HCl.H_2O	59
Compound 145.2HCl.H_2O	62

Experimental Example 11 : Inhibition of angiogenesis

[0156] The inhibition of angiogenesis was evaluated by using the inhibition of lumen formation in vascular endothelial cell as an index. To be specific, normal human umberical vascular endothelial cells (KURABO INDUSTRIES LTD.) were suspended in E-GMUV medium at 5.5×10^4 cells/ml and 400 μl therefrom was added on matrigel plate (EHS sarcoma-derived reconstructed basement membrane, Collaborative Biomedical Products). Then, the test compound (1 mM solution, 4 μl) was added and the cells were incubated at 37°C under 5% CO_2 gas for 18 hours. After the completion of the incubation, the number of lumen per predetermined area was counted under a microscope. Inasmuch as the number of lumen increases by the inhibition of lumen formation, the test compound was evaluated by comparison of the number

of lumen with the control. The results are shown in Table 9.

Table 9

Test compound	Number of lumen (10 μM)
Compound 109.2HCl	153%
Compound 80.2HCl.H_2O	174%
Compound 110.2HCl.H_2O	203%
Compound 165.2HCl.3/2H_2O	222%
Compound 204.2HCl	133%

Experimental Example 12 : Inhibition of growth of vascular smooth muscle cell

[0157] The separation from the artery of rat and culture of smooth muscle cell (SMC) followed the explant method of Ross (Ross, R and Glomset, J. A., N. Engl. J. Med., 295, 369-420, 1976). Male wistar rats (10 week old) was slaughtered by cutting the carotid arteries and aorta of thorax was removed. After removal of fat tissues around the tunica externa and peeling of tunica intima, the artery was minced and incubated in 10% fetal bovine serum (FBS)-containing DMEM medium at 37°C under 5% CO_2 gas. Seven days later, the out-grown cells were separated by trypsin treatment, washed with phosphate-buffered saline (PBS) and incubated in 10% FBS-containing DMEM medium in a 80 cm^2 culture flask. The cells of subculture 2 were suspended in 10% FBS-containing DMEM medium at 5×10^4 cells/ml and 100 μl thereof per well was added to 96 well collagen-coated plate, which was incubated at 37°C under 5% CO_2 gas for one day. The test compound was appropriately diluted with dimethyl sulfoxide (DMSO) and added to the 96 well plate. The concentration of DMSO in the medium was adjusted to 1%. After 48 hours, 10 μl of MTP solution (5 mg/ml) was added and, 4 hours later, 10% sodium dodecyl sulfate-0.01 N hydrochloric acid (50 μl) was added. The absorbance at 570 nm was measured the following day by an immunoreader. The SMC growth inhibitory action of the test compound was shown by inhibition percentage calculated by the following formula The results are shown in Table 10.

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance when test compound was added}}{\text{Absorbance when test compound was not added}}\right) \times 100$$

Table 10

Test compound	IC_{50} (μM)
compound 153.2HCl	27
compound 157.2HCl.H_2O	55
compound 165.2HCl.3/2H_2O	38
compound 163.2HBr	63

Experimental Example 13 : Acute toxicity

[0158] The compound 109.2HCl and compound 143.2HCl.H_2O were respectively administered intraperitoneally to ddY mice and the mice were monitored for 5 days. As a result, the intraperitoneal administration at 30 mg/kg did not cause death.

[0159] The foregoing Formulation Examples and pharmacological experiments reveal that the compounds of the formula (I) and the formula (II) have strong Rho kinase inhibitory action. These Rho kinase inhibitors have vasodilating action, trachea relaxing action, peripheral blood flow increasing action, cell adhesion induction inhibitory action, tumor cell metastasis inhibitory action, bone resorption inhibitory action, mouse allogenic MLR inhibitory activity, tumor cell growth inhibitory action, angiogenesis inhibitory action, vascular smooth muscle cell growth inhibitory action and other various actions. Therefore, they are useful as pharmaceutical agents, particularly, a therapeutic agent of hypertension,

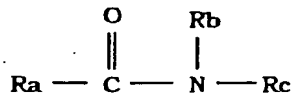
a therapeutic agent of angina pectoris, a suppressive agent of cerebrovascular contraction, a therapeutic agent of asthma, a therapeutic agent of peripheral circulation disorder, a prophylactic agent of immature birth, a therapeutic agent of arteriosclerosis, an anti-cancer drug, an anti-inflammatory agent, an immunosuppressant, a therapeutic agent of autoimmune disease, an anti-AIDS drug, a contraceptive, a prophylactic agent of digestive tract infection, a therapeutic agent of osteoporosis, a therapeutic agent of retinopathy and a brain function improving drug.

[0160] In addition, since Rho kinase inhibitors of the present invention have strong Rho kinase inhibitory activity, they are also useful as reagents for the study relating to Rho and Rho kinase and as diagnostics of the diseases related to them.

[0161] This application is based on application No. 212409/1996 filed in Japan, the contents of which are incorporated hereinto by reference.

Claims

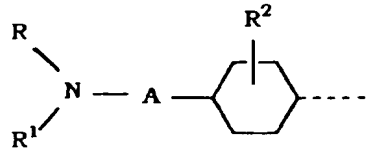
1. A pharmaceutical agent comprising a Rho kinase inhibitor.
2. A therapeutic agent of hypertension, comprising a Rho kinase inhibitor.
3. A therapeutic agent of angina pectoris, comprising a Rho kinase inhibitor.
4. A suppressive agent of cerebrovascular contraction, comprising a Rho kinase inhibitor.
5. A therapeutic agent of asthma, comprising a Rho kinase inhibitor.
6. A therapeutic agent of a peripheral circulation disorder, comprising a Rho kinase inhibitor.
7. A therapeutic agent of arteriosclerosis, comprising a Rho kinase inhibitor.
8. An anti-cancer drug comprising a Rho kinase inhibitor.
9. An anti-inflammatory agent comprising a Rho kinase inhibitor.
10. An immunosuppressant comprising a Rho kinase inhibitor.
11. A therapeutic agent of an autoimmune disease, comprising a Rho kinase inhibitor.
12. An anti-AIDS drug comprising a Rho kinase inhibitor.
13. A therapeutic agent of osteoporosis, comprising a Rho kinase inhibitor.
14. A therapeutic agent of retinopathy, comprising a Rho kinase inhibitor.
15. A brain function improving drug comprising a Rho kinase inhibitor.
16. A prophylactic agent of immature birth, comprising a Rho kinase inhibitor.
17. A contraceptive comprising a Rho kinase inhibitor.
18. A prophylactic agent of digestive tract infection, comprising a Rho kinase inhibitor.
19. A pharmaceutical composition comprising a therapeutically effective amount of a Rho kinase inhibitor and a pharmaceutically acceptable additive.
20. A reagent comprising a Rho kinase inhibitor.
21. A diagnostic comprising a Rho kinase inhibitor.
22. A Rho kinase inhibitor comprising an amide compound of the formula (I)



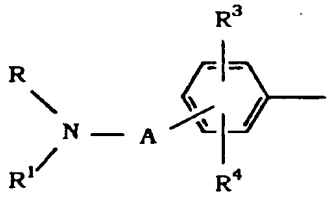
(I)

wherein

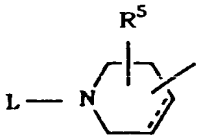
Ra is a group of the formula



(a)



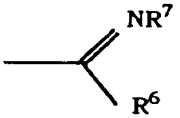
(b) or



(c)

in the formulas (a) and (b),

R is hydrogen, alkyl or cycloalkyl, cycloalkylalkyl, phenyl or aralkyl, which optionally have a substituent on the ring, or a group of the formula



(d)

wherein R^6 is hydrogen, alkyl or formula : $-NR^8NR^9$ wherein R^8 and R^9 are the same or different and each is hydrogen, alkyl, aralkyl or phenyl, R^7 is hydrogen, alkyl, aralkyl, phenyl, nitro or cyano, or R^6 and R^7 in combination show a group forming a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom,

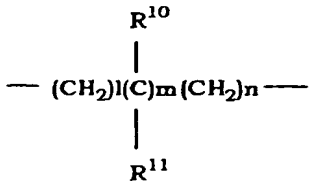
R^1 is hydrogen, alkyl or cycloalkyl, cycloalkylalkyl, phenyl or aralky, which optionally have a substituent on the ring, or

R and R^1 in combination form, together with the adjacent nitrogen atom, a group forming a heterocycle optionally having, in the ring, oxygen atom, sulfur atom or optionally substituted nitrogen atom,

R^2 is hydrogen or alkyl,

R^3 and R^4 are the same or different and each is hydrogen, alkyl, aralkyl, halogen, nitro, amino, alkylamino, acylamino, hydroxy, alkoxy, aralkyloxy, cyano, acyl, mercapto, alkylthio, aralkylthio, carboxy, alkoxycarbonyl, carbamoyl, alkylcarbamoyl or azide, and

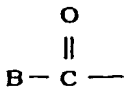
A is a group of the formula



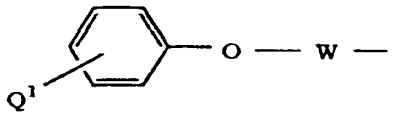
(e)

wherein R^{10} and R^{11} are the same or different and each is hydrogen, alkyl, haloalkyl, aralkyl, hydroxyalkyl, carboxy or alkoxycarbonyl, or R^{10} and R^{11} show a group which forms cycloalkyl in combination and l, m and n are each 0 or an integer of 1-3,
in the formula (c),

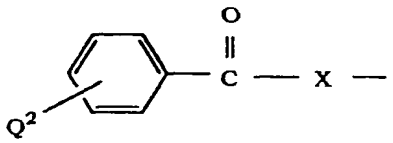
L is hydrogen, alkyl, aminoalkyl, mono or dialkylaminoalkyl, tetrahydrofurfuryl, carbamoylalkyl, phthalimidoalkyl, amidino or a group of the formula



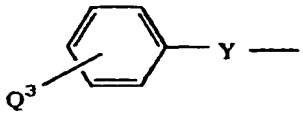
(f)



(g)



(h) or



(i)

wherein B is hydrogen, alkyl, alkoxy, aralkyl, aralkyloxy, aminoalkyl, hydroxyalkyl, alkanoyloxyalkyl, alkoxycarbonylalkyl, α-aminobenzyl, furyl, pyridyl, phenyl, phenylamino, styryl or imidazopyridyl,
Q^1 is hydrogen, halogen, hydroxy, aralkyloxy or thienylmethyl,
W is alkylene,
Q^2 is hydrogen, halogen, hydroxy or aralkyloxy,
X is alkylene,
Q^3 is hydrogen, halogen, hydroxy, alkoxy, nitro, amino, 2,3-dihydrofuryl or 5-methyl-3-oxo-2,3,4,5-tetrahydropyridazin-6-yl; and Y is a single bond, alkylene or alkenylene, and
in the formula (c),

a broken line	is a single bond or a double bond, and
R^5	is hydrogen, hydroxy, alkoxy, alkoxycarbonyloxy, alkanoyloxy or aralkyloxycarbonyloxy;
Rb	is a hydrogen, an alkyl, an aralkyl, an aminoalkyl or a mono or dialkylaminoalkyl; and
Rc	is an optionally substituted heterocycle containing nitrogen, an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

23. A therapeutic agent of hypertension caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

24. A therapeutic agent of angina pectoris caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

25. A suppressive agent of cerebrovascular contraction caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

26. A therapeutic agent of asthma caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

27. A therapeutic agent of peripheral circulation disorder caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

28. A therapeutic agent of arteriosclerosis, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

29. An anti-cancer drug comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

30. An anti-inflammatory agent comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

31. An immunosuppressant comprising a compound of the formula(I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

32. A therapeutic agent of an autoimmune disease, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

33. An anti-AIDS drug comprising a compound of the formula(I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

34. A therapeutic agent of osteoporosis, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

35. A therapeutic agent of retinopathy, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

36. A brain function improving drug comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

37. A prophylactic agent of immature birth, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

38. A contraceptive comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

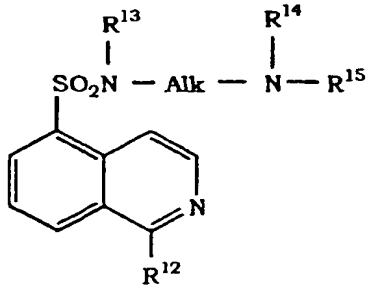
39. A prophylactic agent of digestive tract infection, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

40. A reagent having a Rho kinase inhibitory activity, comprising a compound of the formula (I), an isomer thereof

and/or a pharmaceutically acceptable acid addition salt thereof.

41. A diagnostic of a disease caused by Rho kinase, comprising a compound of the formula (I), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

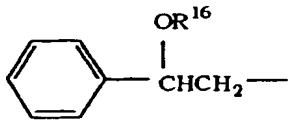
42. A Rho kinase inhibitor containing a substituted isoquinolinesulfonamide derivative of the formula (II)



(II)

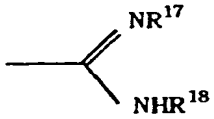
wherein

R^{12} is a hydrogen, a chlorine or a hydroxy, and
when R^{12} is a hydrogen,
Alk is an alkylene having 2 to 6 carbon atoms, which optionally Has alkyl having 1 to 10 carbon atoms, aryl or aralkyl as a substituent;
R^{13} is a hydrogen;
R^{14} is a hydrogen, or a linear or branched alkyl having 1 to 6 carbon atoms, an aryl or an aralkyl;
R^{15} is a hydrogen, a linear or branched alkyl having 1 to 6 carbon atoms, an aryl or an aralkyl, or a benzoyl, a cinnamyl, a cinnamoyl, a furoyl or a group of the following formula



(j)

wherein R^{16} is linear or branched alkyl having 1 to 6 carbon atoms or a group of the following formula



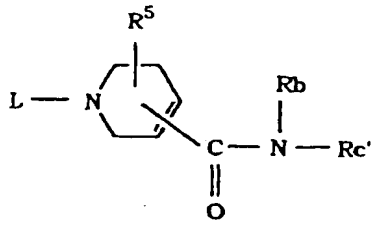
(k)

wherein R^{17} and R^{18} are hydrogen or directly bonded to form alkylene having 2 to 4 carbon atoms; or
R^{13} and R^{14} are directly bonded to form alkylene having 4 or less carbon atoms, which is optionally substituted by alkyl having 1 to 10 carbon atoms, phenyl or benzyl, or
R^{14} and R^{15} directly or in combination via oxygen atom form a heterocycle together with the adjacent nitrogen atom, and
when R^{12} is a chlorine or a hydroxy,
Alk is an alkylene having 2 to 6 carbon atoms, which is optionally substituted at the hydrogen bonded to carbon by alkyl having 1 to 6 carbon atoms,

and/or a pharmaceutically acceptable acid addition salt thereof.

61. A diagnostic of a disease caused by Rho kinase, comprising a compound of the formula (II), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

62. A compound of the formula (III)



(III)

wherein Rc' is an optionally substituted heterocycle having nitrogen, which is other than pyridine of Rc, and other symbols are as defined above, an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof.

63. The pharmaceutical agent of any of claims 1 to 18, comprising a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

64. The pharmaceutical composition of claim 19, comprising a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

65. The reagent of claim 20, comprising a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

66. The diagnostic of claim 21, comprising a compound of the formula (III), an isomer thereof and/or a pharmaceutically acceptable acid addition salt thereof as a Rho kinase inhibitor.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02793

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl^6 A61K45/00, A61K31/16, A61K31/165, A61K31/195, A61K49/00, A61K31/445, A61K31/50, A61K31/495, A61K31/44, C07D213/81, C07D401/12, C07D409/14,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) Int. Cl^6 A61K45/00, A61K31/16, A61K31/165, A61K31/195, A61K49/00, A61K31/445, A61K31/50, A61K31/495, A61K31/44, C07D213/81, C07D401/12,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN), REGISTRY(STN), WPIDS(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP, 62-89679, A (Yoshitomi Pharmaceutical Industries, Ltd.), April 24, 1987 (24. 04. 87), Pages 1, 2 (Family: none)	1, 2, 4, 19, 22, 23, 25
X	JP, 3-218356, A (Yoshitomi Pharmaceutical Industries, Ltd.), September 25, 1991 (25. 09. 91), Pages 1 to 3 (Family: none)	1, 2, 4, 19, 22, 23, 25
X	JP, 4-273821, A (Yoshitomi Pharmaceutical Industries, Ltd.), September 30, 1992 (30. 09. 92), Abstract (Family: none)	1, 5, 19, 22, 26
X	JP, 5-194401, A (Yoshitomi Pharmaceutical Industries, Ltd.), August 3, 1993 (03. 08. 93), Abstract & WO, 9305021, A	1, 5, 19, 22, 26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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November 4, 1997 (04. 11. 97)	November 18, 1997 (18. 11. 97)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02793

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP, 6-41080, A (Yoshitomi Pharmaceutical Industries, Ltd.), February 15, 1994 (15. 02. 94), Abstract & WO, 9305021, A	1, 4, 5, 19, 22, 25, 26
X	JP, 57-200366, A (Asahi Chemical Industry Co., Ltd. and another), December 8, 1982 (08. 12. 82), Pages 1, 2 & EP, 61673, A	1, 3, 4, 6, 19, 42, 44, 45
X	JP, 61-227581, A (Asahi Chemical Industry Co., Ltd.), October 9, 1986 (09. 10. 86), Page 1 & EP, 187371, A	1, 2, 3, 4, 19, 42, 43, 44, 45
X	JP, 2-256617, A (Asahi Chemical Industry Co., Ltd.), October 17, 1990 (17. 10. 90), Page 1 (Family: none)	1, 15, 19, 42, 56
X	JP, 4-264030, A (Asahi Chemical Industry Co., Ltd.), September 18, 1992 (18. 09. 92), Abstract (Family: none)	1, 5, 19, 42, 46
X	JP, 6-56668, A (Asahi Chemical Industry Co., Ltd.), March 1, 1994 (01. 03. 94), Abstract & WO, 9403171, A	1, 19, 42
X	JP, 6-80569, A (Asahi Chemical Industry Co., Ltd.), March 22, 1994 (22. 03. 94), Abstract & WO, 9405290, A	1, 6, 19, 42, 47
X	JP, 6-293643, A (Asahi Chemical Industry Co., Ltd.), October 21, 1994 (21. 10. 94), Abstract (Family: none)	1, 19, 42
X	JP, 7-41424, A (Asahi Chemical Industry Co., Ltd.), February 10, 1995 (10. 02. 95), Abstract (Family: none)	1, 9, 19, 42, 50
X	JP, 7-277979, A (Asahi Chemical Industry Co., Ltd.), October 24, 1995 (24. 10. 95), Abstract (Family: none)	1, 19, 42
X	WO, 9528387, A (Yoshitomi Pharmaceutical Industries, Ltd.), October 26, 1995 (26. 10. 95), Pages 1, 2 & EP, 757038, A	1, 2, 3, 4, 6, 19, 22, 23, 24, 25, 27

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/54 — A1

(11) International Publication Number: WO 97/15308

(43) International Publication Date: 1 May 1997 (01.05.97)

(21) International Application Number: PCT/US96/17019

(22) International Filing Date: 23 October 1996 (23.10.96)

(30) Priority Data:
60/005,830 23 October 1995 (23.10.95) US

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(81) Designated States: AL, AM, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS AND METHODS FOR TREATING BONE DEFICIT CONDITIONS

(57) Abstract

Compounds containing two aromatic systems covalently linked through a linker containing one or more atoms, or "linker" defined as including a covalent bond *per se* so as to space the aromatic systems at a distance 1.5-15Å, are effective in treating conditions associated with bone deficits. The compounds can be administered to vertebrate subjects alone or in combination with additional agents that promote bone growth or that inhibit bone resorption. They can be screened for activity prior to administration by assessing their ability to effect the transcription of a reporter gene coupled to a promoter associated with a bone morphogenetic protein and/or their ability to stimulate calvarial growth in model animal systems.

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COMPOSITIONS AND METHODS FOR TREATING BONE DEFICIT CONDITIONS

Technical Field

The invention relates to compositions and methods for use in limiting undesired bone loss in a vertebrate at risk of such bone loss, in treating conditions that are characterized by undesired bone loss or by the need for bone growth, in treating fractures, and in treating cartilage disorders. More specifically, the invention concerns the use of specific classes of compounds identified or characterized by a high throughput screening assay.

Background Art

Bone is not a static tissue. It is subject to constant breakdown and resynthesis in a complex process mediated by osteoblasts, which produce new bone, and osteoclasts, which destroy bone. The activities of these cells are regulated by a large number of cytokines and growth factors, many of which have now been identified and cloned. Mundy has described the current knowledge related to these factors (Mundy, G.R. *Clin Orthop* 324:24-28, 1996; Mundy, G.R. *J Bone Miner Res* 8:S505-10, 1993).

Although there is a great deal of information available on the factors which influence the breakdown and resorption of bone, information on growth factors which stimulate the formation of new bone is more limited. Investigators have searched for sources of such activities, and have found that bone tissue itself is a storehouse for factors which have the capacity for stimulating bone cells. Thus, extracts of bovine bone tissue obtained from slaughterhouses contain not only structural proteins which are responsible for maintaining the structural integrity of bone, but also biologically active bone growth factors which can stimulate bone cells to proliferate. Among these latter factors are transforming growth factor ß, the heparin-binding growth factors (acidic and basic fibroblast growth factor), the insulin-like growth factors (insulin-like growth factor I and insulin-like growth factor II), and a recently described family of proteins called bone morphogenetic proteins (BMPs). All of these growth factors have effects on other types of cells, as well as on bone cells.

The BMPs are novel factors in the extended transforming growth factor ß superfamily. They were first identified by Wozney J. *et al. Science* (1988) 242:1528-34, using gene cloning techniques, following earlier descriptions characterizing the biological activity in extracts of demineralized bone (Urist M. *Science* (1965) 150:893-99). Recombinant BMP2 and BMP4 can induce new bone formation when they are injected locally into the subcutaneous tissues of rats (Wozney J. *Molec Reprod Dev* (1992) 32:160-67). These factors are expressed by normal osteoblasts as they differentiate, and have been shown to stimulate osteoblast differentiation and bone nodule formation *in vitro* as well as bone formation *in vivo* (Harris S. *et al. J. Bone Miner Res* (1994) 9:855-63). This latter property suggests potential usefulness as therapeutic agents in diseases which result in bone loss.

The cells which are responsible for forming bone are osteoblasts. As osteoblasts differentiate from precursors to mature bone-forming cells, they express and secrete a number of enzymes and structural proteins of the bone matrix, including Type-1 collagen, osteocalcin, osteopontin and alkaline phosphatase (Stein G. *et al. Curr Opin Cell Biol* (1990) 2:1018-27; Harris S. *et al.* (1994), *supra*). They also synthesize a number of growth regulatory peptides which are stored in the bone matrix, and are presumably responsible for normal bone formation. These growth regulatory peptides include the BMPs (Harris S. *et al.* (1994), *supra*). In studies of primary cultures of fetal rat calvarial osteoblasts, BMPs 1, 2, 3, 4, and 6 are expressed by cultured cells prior to the formation of mineralized bone nodules (Harris S. *et al.* (1994), *supra*). Like alkaline phosphatase, osteocalcin and osteopontin, the BMPs are expressed by cultured osteoblasts as they proliferate and differentiate.

Although the BMPs are potent stimulators of bone formation *in vitro* and *in vivo*, there are disadvantages to their use as therapeutic agents to enhance bone healing. Receptors for the bone morphogenetic proteins have been identified in many tissues, and the BMPs themselves are expressed in a large variety of tissues in specific temporal and spatial patterns. This suggests that BMPs may have effects on many tissues other than bone, potentially limiting their usefulness as therapeutic agents when administered systemically. Moreover, since they are peptides, they would have to be administered by injection. These disadvantages impose severe limitations to the development of BMPs as therapeutic agents.

There is a plethora of conditions which are characterized by the need to enhance bone formation. Perhaps the most obvious is the case of bone fractures, where it would be desirable to stimulate bone growth and to hasten and complete bone repair. Agents that enhance bone formation would also be useful in facial reconstruction procedures. Other bone deficit conditions include bone segmental defects, periodontal disease, metastatic bone disease, osteolytic bone disease and conditions where connective tissue repair would be beneficial, such as healing or regeneration of cartilage defects or injury. Also of great significance is the chronic condition of osteoporosis, including age-related osteoporosis and osteoporosis associated with post-menopausal hormone status. Other conditions characterized by the need for bone growth include primary and secondary hyperparathyroidism, disuse osteoporosis, diabetes-related osteoporosis, and glucocorticoid-related osteoporosis. In addition, or alternatively, the compounds of the present invention may modulate metabolism, proliferation and/or differentiation of normal or aberrant cells or tissues.

There are currently no satisfactory pharmaceutical approaches to managing any of these conditions. Bone fractures are still treated exclusively using casts, braces, anchoring devices and other strictly mechanical means. Further bone deterioration associated with post-menopausal osteoporosis has been decreased or prevented with estrogens or bisphosphonates.

US Patent 5, 280, 040 discloses a class of compounds which are 3, 4-diaryl chromans. These compounds can be considered derivatives of 2,3,4 triphenyl butanol, where the hydroxy at the 1-position forms an ether with the ortho position of the phenyl group substituted at the 4-position of the butanol. The parent 3,4-diaryl chromans do not contain nitrogen atoms in the aromatic moieties or their linkers. A preferred compound, centchroman, contains a nitrogen substituent only in one of the substituents on a phenyl moiety. These compounds are disclosed in the '040 patent as useful in the treatment of osteoporosis.

The present invention discloses compounds useful for limiting or treating bone deficit conditions, and for other uses that should be apparent to those skilled in the art from the teachings herein.

Disclosure of the Invention

The invention provides compounds that can be administered as ordinary pharmaceuticals and have the metabolic effect of enhancing bone growth. The compounds of the invention can be identified using an assay for their ability to activate control elements associated with these factors. Thus, the invention is directed to methods and compositions for stimulating the growth of skeletal (bone) tissue, which methods and compositions use, as active ingredients, compounds wherein two aromatic systems are coupled so as to be spaced apart from each other by about 1.5 to about 15 Angstroms. The thus-linked systems (including the linker coupling them) may include at least one nitrogen atom other than a ring substituent.

Therefore, the compounds useful in the invention can be described as having the formula Ar^1-linker-Ar^2, wherein each of Ar^1 and Ar^2 is independently an aromatic system and the linker portion of the formula spaces Ar^1 and Ar^2 apart by a distance of approximately 1.5-15 Angstroms. Ar^1, Ar^2 and the linker may optionally be substituted with non interfering substituents. In the useful compounds, there may be at least one nitrogen atom in either Ar^1, Ar^2 and/or the linker, independent of any substituents thereon. Preferably, the compounds of the invention also contain at least one additional heteroatom selected from the group consisting of N, S and O, independent of any substituent.

Other compounds of the invention include particular five membered rings having charge separation.

Thus, the invention is directed to methods to treat bone disorders using the compounds described and to pharmaceutical compositions for this use.

Brief Description of the Drawings

Figure 1 shows the dose response curve for the compound, designated 59-0008.

Figures 2 and 3 show illustrative compounds of the invention and the results obtained with them in an *in vitro* test.

Modes of Carrying Out the Invention

A rapid throughput screening test for compounds capable of stimulating expression of a reporter gene linked to a BMP promoter (a surrogate for the production of bone morphogenetic factors that are endogenously produced) is described in U.S. Application

Serial No. 08/458,434, filed 2 June 1995, the entire contents of which are incorporated herein by reference. This assay is also described as a portion of a study of immortalized murine osteoblasts (derived from a mouse expressing a transgene composed of a BMP2 promoter driving expression of T-antigen) in Ghosh-Choudhery, N. *et al. Endocrinology* (1996) 137:331-39. In this study, the immortalized cells were stably transfected with a plasmid containing a luciferase reporter gene driven by a mouse BMP2 promoter (-2736/114 bp), and responded in a dose-dependent manner to recombinant human BMP2.

Briefly, the assay utilizes cells transformed permanently or transiently with constructs in which the promoter of a bone morphogenetic protein, specifically BMP2 or BMP4, is coupled to a reporter gene, typically luciferase. These transformed cells are then evaluated for the production of the reporter gene product; compounds that activate the BMP promoter will drive production of the reporter protein, which can be readily assayed. Over 40,000 compounds have been subjected to this rapid screening technique, and only a very small percentage are able to elicit a level of production of luciferase 5-fold greater than that produced by vehicle. Compounds that activate the BMP promoter share certain structural characteristics not present in inactive compounds. The active compounds ("BMP promoter-active compounds" or "active compounds") are useful in promoting bone or cartilage growth, and thus in the treatment of vertebrates in need of bone or cartilage growth.

BMP promoter-active compounds can be examined in a variety of other assays that test specificity and toxicity. For instance, non-BMP promoters or response elements can be linked to a reporter gene and inserted into an appropriate host cell. Cytotoxicity can be determined by visual or microscopic examination of BMP promoter- and/or non-BMP promoter-reporter gene-containing cells, for instance. Alternatively, nucleic acid and/or protein synthesis by the cells can be monitored. For *in vivo* assays, tissues may be removed and examined visually or microscopically, and optionally examined in conjunction with dyes or stains that facilitate histologic examination. In assessing *in vivo* assay results, it may also be useful to examine biodistribution of the test compound, using conventional medicinal chemistry/animal model techniques.

As used herein, "limit" or "limiting" and "treat" or "treatment" are interchangeable terms. The terms include a postponement of development of bone deficit symptoms and/or a reduction in the severity of such symptoms that will or are expected to develop. The

terms further include ameliorating existing bone or cartilage deficit symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, preventing or reversing bone resorption and/or encouraging bone growth. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with a cartilage, bone or skeletal deficit, or with the potential to develop such deficit.

By "bone deficit" is meant an imbalance in the ratio of bone formation to bone resorption, such that, if unmodified, the subject will exhibit less bone than desirable, or the subject's bones will be less intact and coherent than desired. Bone deficit may also result from fracture, from surgical intervention or from dental or periodontal disease. By "cartilage defect" is meant damaged cartilage, less cartilage than desired, or cartilage that is less intact and coherent than desired.

Representative uses of the compounds of the present invention include: repair of bone defects and deficiencies, such as those occuring in closed, open and non-union fractures; prophylactic use in closed and open fracture reduction; promotion of bone healing in plastic surgery; stimulation of bone ingrowth into non-cemented prosthetic joints and dental implants; elevation of peak bone mass in pre-menopausal women; treatment of growth deficiencies; treatment of peridontal disease and defects, and other tooth repair processes; increase in bone formation during distraction osteogenesis; and treatment of other skeletal disorders, such as age-related osteoporosis, post-menopausal osteoporosis, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis. The compounds of the present invention can also be useful in repair of congenital, trauma-induced or surgical resection of bone (for instance, for cancer treatment), and in cosmetic surgery Further, the compounds of the present invention can be used for limiting or treating cartilage defects or disorders, and may be useful in wound healing or tissue repair.

Bone or cartilage deficit or defect can be treated in vertebrate subjects by administering compounds of the invention which exhibit certain structural and functional characteristics. The compositions of the invention may be administered systemically or locally. For systemic use, the compounds herein are formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intraperitoneal, intranasal or transdermal) or enteral (e.g., oral or rectal) delivery according to conventional methods. Intravenous administration can be by a series of injections or by continuous infusion over an extended period. Administration by injection or other routes of discretely spaced administration can

be performed at intervals ranging from weekly to once to three times daily. Alternatively, the compounds disclosed herein may be administered in a cyclical manner (administration of disclosed compound; followed by no administration; followed by administration of disclosed compound, and the like). Treatment will continue until the desired outcome is achieved. In general, pharmaceutical formulations will include a compound of the present invention in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water, borate-buffered saline containing trace metals or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, lubricants, fillers, stabilizers, etc. Methods of formulation are well known in the art and are disclosed, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton PA, 1990, which is incorporated herein by reference. Pharmaceutical compositions for use within the present invention can be in the form of sterile, non-pyrogenic liquid solutions or suspensions, coated capsules, suppositories, lyophilized powders, transdermal patches or other forms known in the art. Local administration may be by injection at the site of injury or defect, or by insertion or attachment of a solid carrier at the site, or by direct, topical application of a viscous liquid, or the like. For local administration, the delivery vehicle preferably provides a matrix for the growing bone or cartilage, and more preferably is a vehicle that can be absorbed by the subject without adverse effects.

Delivery of compounds herein to wound sites may be enhanced by the use of controlled-release compositions, such as those described in pending U.S. Patent Application No. 07/871,246 (corresponding to WIPO publication WO 93/20859, which is incorporated herein by reference in its entirety). Films of this type are particularly useful as coatings for prosthetic devices and surgical implants. The films may, for example, be wrapped around the outer surfaces of surgical screws, rods, pins, plates and the like. Implantable devices of this type are routinely used in orthopedic surgery. The films can also be used to coat bone filling materials, such as hydroxyapatite blocks, demineralized bone matrix plugs, collagen matrices and the like. In general, a film or device as described herein is applied to the bone at the fracture site. Application is generally by implantation into the bone or attachment to the surface using standard surgical procedures.

In addition to the copolymers and carriers noted above, the biodegradable films and matrices may include other active or inert components. Of particular interest are those

agents that promote tissue growth or infiltration, such as growth factors. Exemplary growth factors for this purpose include epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factors (TGFs), parathyroid hormone (PTH), leukemia inhibitory factor (LIF), and insulin-like growth factors (IGFs) and the like. Agents that promote bone growth, such as bone morphogenetic proteins (U.S. Patent No. 4,761,471; PCT Publication WO 90/11366), osteogenin (Sampath *et al. Proc. Natl. Acad. Sci. USA* (1987) 84:7109-13) and NaF (Tencer *et al. J. Biomed. Mat. Res.* (1989) 23: 571-89) are also preferred. Biodegradable films or matrices include calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyanhydrides, bone or dermal collagen, pure proteins, extracellular matrix components and the like and combinations thereof. Such biodegradable materials may be used in combination with non-biodegradable materials, to provide desired mechanical, cosmetic or tissue or matrix interface properties.

Alternative methods for delivery of compounds of the present invention include use of ALZET osmotic minipumps (Alza Corp., Palo Alto, CA); sustained release matrix materials such as those disclosed in Wang *et al.* (PCT Publication WO 90/11366); electrically charged dextran beads, as disclosed in Bao *et al.* (PCT Publication WO 92/03125); collagen-based delivery systems, for example, as disclosed in Ksander *et al. Ann. Surg.* (1990) 211(3):288-94; methylcellulose gel systems, as disclosed in Beck *et al. J. Bone Min. Res.* (1991) 6(11):1257-65; and alginate-based systems, as disclosed in Edelman *et al. Biomaterials* (1991) 12:619-26 and the like. Other methods well known in the art for sustained local delivery in bone include porous coated metal protheses that can be impregnated and solid plastic rods with therapeutic compositions incorporated within them.

The compounds of the present invention may also be used in conjunction with agents that inhibit bone resorption. Antiresorptive agents, such as estrogen, bisphosphonates and calcitonin, are preferred for this purpose. More specifically, the compounds disclosed herein may be administered for a period of time (for instance, months to years) sufficient to obtain correction of a bone deficit condition. Once the bone deficit condition has been corrected, the vertebrate can be administered an anti-resorptive compound to maintain the corrected bone condition. Alternatively, the compounds disclosed herein may be adminstered with an anti-resorptive compound in a cyclical manner

(administration of disclosed compound, followed by anti-resorptive, followed by disclosed compound, and the like).

In additional formulations, conventional preparations such as those described below may be used.

Aqueous suspensions may contain the active ingredient in admixture with pharmacologically acceptable excipients, comprising suspending agents, such as methyl cellulose; and wetting agents, such as lecithin, lysolecithin or long-chain fatty alcohols. The said aqueous suspensions may also contain preservatives, coloring agents, flavoring agents and sweetening agents in accordance with industry standards.

Preparations for topical and local application comprise aerosol sprays, lotions, gels and ointments in pharmaceutically appropriate vehicles which may comprise lower aliphatic alcohols, polyglycols such as glycerol, polyethylene glycol, esters of fatty acids, oils and fats, and silicones. The preparations may further comprise antioxidants, such as ascorbic acid or tocopherol, and preservatives, such as p-hydroxybenzoic acid esters.

Parenteral preparations comprise particularly sterile or sterilized products. Injectable compositions may be provided containing the active compound and any of the well known injectable carriers. These may contain salts for regulating the osmotic pressure.

If desired, the osteogenic agents can be incorporated into liposomes by any of the reported methods of preparing liposomes for use in treating various pathogenic conditions. The present compositions may utilize the compounds noted above incorporated in liposomes in order to direct these compounds to macrophages, monocytes, other cells and tissues and organs which take up the liposomal composition. The liposome-incorporated compounds of the invention can be utilized by parenteral administration, to allow for the efficacious use of lower doses of the compounds. Ligands may also be incorporated to further focus the specificity of the liposomes.

Suitable conventional methods of liposome preparation include, but are not limited to, those disclosed by Bangham, A.D. *et al. J Mol Biol* (1965) 23:238-252, Olson, F. *et al. Biochim Biophys Acta* (1979) 557:9-23, Szoka, F. *et al. Proc Natl Acad Sci USA* (1978) 75:4194-4198, Mayhew, E. *et al.* _____ (1984) 775:169175, Kim, S. *et al. Biochim Biophys Acta* (1983) 728:339:348, and Mayer, *et al. Biochim Biophys Acta* (1986) 858:161-168.

The liposomes may be made from the present compounds in combination with any of the conventional synthetic or natural phospholipid liposome materials including phospholipids from natural sources such as egg, plant or animal sources such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, sphingomyelin, phosphatidylserine, or phosphatidylinositol. Synthetic phospholipids that may also be used, include, but are not limited to: dimyristoylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidycholine, and the corresponding synthetic phosphatidylethanolamines and phosphatidylglycerols. Cholesterol or other sterols, cholesterol hemisuccinate, glycolipids, cerebrosides, fatty acids, gangliosides, sphingolipids, 1,2-bis(oleoyloxy)-3-(trimethyl ammonio) propane (DOTAP), N-[1-(2,3-dioleoyl) propyl-N,N,N-trimethylammonium chloride (DOTMA), and other cationic lipids may be incorporated into the liposomes, as is known to those skilled in the art. The relative amounts of phospholipid and additives used in the liposomes may be varied if desired. The preferred ranges are from about 60 to 90 mole percent of the phospholipid; cholesterol, cholesterol hemisuccinate, fatty acids or cationic lipids may be used in amounts ranging from 0 to 50 mole percent. The amounts of the present compounds incorporated into the lipid layer of liposomes can be varied with the concentration of the lipids ranging from about 0.01 to about 50 mole percent.

Using conventional methods, approximately 20 to 30% of the compound present in solution can be entrapped in liposomes; thus, approximately 70 to 80% of the active compound is wasted. In contrast, where the compound is incorporated into liposomes, virtually all of the compound is incorporated into the liposome, and essentially none of the active compound is wasted.

The liposomes with the above formulations may be made still more specific for their intended targets with the incorporation of monoclonal antibodies or other ligands specific for a target. For example, monoclonal antibodies to the BMP receptor may be incorporated into the liposome by linkage to phosphatidylethanolamine (PE) incorporated into the liposome by the method of Leserman, L. *et al. Nature* (1980) 288:602-604.

Veterinary uses of the disclosed compounds are also contemplated. Such uses would include limitation or treatment of bone or cartilage deficits or defects in domestic animals, livestock and thoroughbred horses. The compounds described herein can also

modify a target tissue or organ environment, so as to attract bone-forming cells to an environment in need of such cells.

The compounds of the present invention may also be used to stimulate growth of bone-forming cells or their precursors, or to induce differentiation of bone-forming cell precursors, either *in vitro* or *ex vivo*. As used herein, the term "precursor cell" refers to a cell that is committed to a differentiation pathway, but that generally does not express markers or function as a mature, fully differentiated cell. As used herein, the term "mesenchymal cells" or "mesenchymal stem cells" refers to pluripotent progenitor cells that are capable of dividing many times, and whose progeny will give rise to skeletal tissues, including cartilage, bone, tendon, ligament, marrow stroma and connective tissue (see A. Caplan *J. Orthop. Res.* (1991) 9:641-50). As used herein, the term "osteogenic cells" includes osteoblasts and osteoblast precursor cells. More particularly, the disclosed compounds are useful for stimulating a cell population containing marrow mesenchymal cells, thereby increasing the number of osteogenic cells in that cell population. In a preferred method, hematopoietic cells are removed from the cell population, either before or after stimulation with the disclosed compounds. Through practice of such methods, osteogenic cells may be expanded. The expanded osteogenic cells can be infused (or reinfused) into a vertebrate subject in need thereof. For instance, a subject's own mesenchymal stem cells can be exposed to compounds of the present invention *ex vivo*, and the resultant osteogenic cells could be infused or directed to a desired site within the subject, where further proliferation and/or differentiation of the osteogenic cells can occur without immunorejection. Alternatively, the cell population exposed to the disclosed compounds may be immortalized human fetal osteoblastic or osteogenic cells. If such cells are infused or implanted in a vertebrate subject, it may be advantageous to "immunoprotect" these non-self cells, or to immunosuppress (preferably locally) the recipient to enhance transplantation and bone or cartilage repair.

Within the present invention, an "effective amount" of a composition is that amount which produces a statistically significant effect. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an active compound herein required to provide a clinically significant increase in healing rates in fracture repair; reversal of bone loss in osteoporosis; reversal of cartilage defects or disorders; prevention or delay of onset of osteoporosis; stimulation and/or augmentation of bone formation in

fracture non-unions and distraction osteogenesis; increase and/or acceleration of bone growth into prosthetic devices; and repair of dental defects. Such effective amounts will be determined using routine optimization techniques and are dependent on the particular condition to be treated, the condition of the patient, the route of administration, the formulation, and the judgment of the practitioner and other factors evident to those skilled in the art. The dosage required for the compounds of the invention (for example, in osteoporosis where an increase in bone formation is desired) is manifested as a statistically significant difference in bone mass between treatment and control groups. This difference in bone mass may be seen, for example, as a 5-20% or more increase in bone mass in the treatment group. Other measurements of clinically significant increases in healing may include, for example, tests for breaking strength and tension, breaking strength and torsion, 4-point bending, increased connectivity in bone biopsies and other biomechanical tests well known to those skilled in the art. General guidance for treatment regimens is obtained from experiments carried out in animal models of the disease of interest.

The dosage of the compounds of the invention will vary according to the extent and severity of the need for treatment, the activity of the administered compound, the general health of the subject, and other considerations well known to the skilled artisan. Generally, they can be administered to a typical human on a daily basis on an oral dose of about 0.1 mg/kg-1000 mg/kg, and more preferably from about 1 mg/kg to about 200 mg/kg. The parenteral dose will appropriately be 20-100% of the oral dose.

Screening Assays

The osteogenic activity of the compounds used in the methods of the invention can be verified using *in vitro* screening techniques, such as the assessment of transcription of a reporter gene coupled to a bone morphogenetic protein-associated promoter, as described above, or in alternative assays such as the following:

Technique for Neonatal Mouse Calvaria Assay (*In vitro*)

This assay is similar to that described by Gowen M. & Mundy G. *J Immunol* (1986) 136:2478-82. Briefly, four days after birth, the front and parietal bones of ICR Swiss white mouse pups are removed by microdissection and split along the sagittal suture. The bones are incubated in BGJb medium (Irvine Scientific, Santa Ana, CA) plus 0.02% (or lower

concentration) β-methylcyclodextrin, wherein the medium also contains test or control substances, at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 96 hours.

Following this, the bones are removed from the incubation media and fixed in 10% buffered formalin for 24-48 hours, decalcified in 14% EDTA for 1 week, processed through graded alcohols; and embedded in paraffin wax. Three μm sections of the calvaria are prepared. Representative sections are selected for histomorphometric assessment of bone formation and bone resorption. Bone changes are measured on sections cut 200 μm apart. Osteoblasts and osteoclasts are identified by their distinctive morphology.

Other auxillary assays can be used as controls to determine non-BMP promoter-mediated effects of test compounds. For example, mitogenic activity can be measured using screening assays featuring a serum-response element (SRE) as a promoter and a luciferase reporter gene. More specifically, these screening assays can detect signalling through SRE-mediated pathways, such as the protein kinase C pathway. For instance, an osteoblast activator SRE-luciferase screen and an insulin mimetic SRE-luciferase screen are useful for this purpose. Similarly, test compound stimulation of cAMP response element (CRE)-mediated pathways can also be assayed. For instance, cells transfected with receptors for PTH and calcitonin (two bone-active agents) can be used in CRE-luciferase screens to detect elevated cAMP levels. Thus, the BMP promoter specificity of a test compound can be examined through use of these types of auxillary assays.

In vivo Assay of Effects of Compounds on Murine Calvarial Bone Growth

Male ICR Swiss white mice, aged 4-6 weeks and weighing 13-26 gm, are employed, using 4-5 mice per group. The calvarial bone growth assay is performed as described in PCT application WO 95/24211, incorporated by reference. Briefly, the test compound or appropriate control vehicle is injected into the subcutaneous tissue over the right calvaria of normal mice. Typically, the control vehicle is the vehicle in which the compound was solubilized, and is PBS containing 5% DMSO or is PBS containing Tween (2 μl/10 ml). The animals are sacrificed on day 14 and bone growth measured by histomorphometry. Bone samples for quantitation are cleaned from adjacent tissues and fixed in 10% buffered formalin for 24-48 hours, decalcified in 14% EDTA for 1-3 weeks, processed through graded alcohols; and embedded in paraffin wax. Three to five μm

sections of the calvaria are prepared, and representative sections are selected for histomorphometric assessment of the effects on bone formation and bone resorption. Sections are measured by using a camera lucida attachment to trace directly the microscopic image onto a digitizing plate. Bone changes are measured on sections cut 200 μm apart, over 4 adjacent 1x1 mm fields on both the injected and noninjected sides of the calvaria. New bone is identified by its characteristic woven structure, and osteoclasts and osteoblasts are identified by their distinctive morphology. Histomorphometry software (OsteoMeasure, Osteometrix, Inc., Atlanta) is used to process digitizer input to determine cell counts and measure areas or perimeters.

Additional *In Vivo* Assays

Lead compounds can be further tested in intact animals using an *in vivo*, dosing assay. Prototypical dosing may be accomplished by subcutaneous, intraperitoneal or oral administration, and may be performed by injection, sustained release or other delivery techniques. The time period for administration of test compound may vary (for instance, 28 days as well as 35 days may be appropriate). An exemplary, *in vivo* subcutaneous dosing assay may be conducted as follows:

In a typical study, 70 three-month-old female Sprague-Dawley rats are weight-matched and divided into seven groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; a control group administered vehicle only; a PBS-treated control group; and a positive control group administered a compound (non-protein or protein) known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups.

Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. All animals are injected with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day). Weekly body weights are determined. At the end of the 35-day cycle, the animals are weighed and bled by orbital or cardiac puncture. Serum calcium, phosphate, osteocalcin, and CBCs are determined. Both leg bones (femur and tibia) and lumbar vertebrae are removed, cleaned of adhering soft tissue, and stored in 70% ethanol for evaluation, as performed by peripheral quantitative computed tomography (pQCT; Ferretti,

J. *Bone* (1995) 17:353S-64S), dual energy X-ray absorptiometry (DEXA; Laval-Jeantet A. *et al. Calcif Tissue Intl* (1995) 56:14-18; J. Casez *et al. Bone and Mineral* (1994) 26:61-68) and/or histomorphometry. The effect of test compounds on bone remodeling can thus be evaluated.

Lead compounds can also be tested in acute ovariectomized animals (prevention model) using an *in vivo* dosing assay. Such assays may also include an estrogen-treated group as a control. An exemplary subcutaneous dosing assay is performed as follows:

In a typical study, 80 three-month-old female Sprague-Dawley rats are weight-matched and divided into eight groups, with ten animals in each group. This includes a baseline control group of animals sacrificed at the initiation of the study; three control groups (sham ovariectomized (sham OVX) + vehicle only; ovariectomized (OVX) + vehicle only; PBS-treated OVX); and a control OVX group that is administered a compound known to promote bone growth. Three dosage levels of the compound to be tested are administered to the remaining three groups of OVX animals.

Since ovariectomy (OVX) induces hyperphagia, all OVX animals are pair-fed with sham OVX animals throughout the 35 day study. Briefly, test compound, positive control compound, PBS, or vehicle alone is administered subcutaneously once per day for 35 days. Alternatively, test compound can be formulated in implantable pellets that are implanted for 35 days, or may be administered orally, such as by gastric gavage. All animals, including sham OVX/vehicle and OVX/vehicle groups, are injected intraperitoneally with calcein nine days and two days before sacrifice (two injections of calcein administered each designated day, to ensure proper labeling of newly formed bone). Weekly body weights are determined. At the end of the 35-day cycle, the animals' blood and tissues are processed as described above.

Lead compounds may also be tested in chronic OVX animals (treatment model). An exemplary protocol for treatment of established bone loss in ovariectomized animals that can be used to assess efficacy of anabolic agents may be performed as follows. Briefly, 80 to 100 six month old female, Sprague-Dawley rats are subjected to sham surgery (sham OVX) or ovariectomy (OVX) at time 0, and 10 rats are sacrificed to serve as baseline controls. Body weights are recorded weekly during the experiment. After approximately 6 weeks of bone depletion (42 days), 10 sham OVX and 10 OVX rats are randomly selected for sacrifice as depletion period controls. Of the remaining animals, 10 sham OVX and 10

OVX rats are used as placebo-treated controls. The remaining OVX animals are treated with 3 to 5 doses of test drug for a period of 5 weeks (35 days). As a postitive control, a group of OVX rats can be treated with an agent such as PTH, a known anabolic agent in this model (Kimmel *et al. Endocrinology* (1993) 132:1577-84). To determine effects on bone formation, the following procedure can be followed. The femurs, tibiae and lumbar vertebrae 1 to 4 are excised and collected. The proximal left and right tibiae are used for pQCT measurements, cancellous bone mineral density (BMD) (gravimetric determination), and histology, while the midshaft of each tibiae is subjected to cortical BMD or histology. The femurs are prepared for pQCT scanning of the midshaft prior to biomechanical testing. With respect to lumbar vertebrae (LV), LV2 are processed for BMD (pQCT may also be performed); LV3 are prepared for undecalcified bone histology; and LV4 are processed for mechanical testing.

Nature of the Compounds Useful in the Invention

All of the compounds of the invention contain two aromatic systems, Ar^1 and Ar^2, spaced apart by a linker at a distance of 1.5-15Å, and may contain at least one nitrogen atom. Both the systems represented by Ar^1 and Ar^2 may contain non-interfering substituents. The non-interfering substituents on the aromatic system represented by Ar^1 and the non-interfering substituents on the aromatic system represented by Ar^2 are represented in the formulae herein by R^a and R^b, respectively; however, it is recognized that the designation of one Ar as Ar^1 and the other as Ar^2 is arbitrary. For ease of reference, each is designated separately; it will, however, be evident that the linkers described below, unless palindromic, could thus exist in the compounds in "reverse" order of atoms. Generally, the non-interfering substituents can be of wide variety. Among substituents that do not interfere with the beneficial effect of the compounds of the invention on bone in treated subjects are included alkyl (1-6C, preferably lower alkyl 1-4C), including straight or branched-chain forms thereof, alkenyl (1-6C, preferably 1-4C), alkynyl (1-6C, preferably 1-4C), all of which can be straight or branched chains and may contain further substituents; halogens, including F, Cl, Br and I; siloxy, OR, SR, NR_2, OOCR, COOR, NCOR, NCOOR, and benzoyl, CF_3, OCF_3, SCF_3, $N(CF_3)_2$, CN, SO, SO_2R and SO_3R wherein R is alkyl (1-6C) or is H. Where two R^a or two R^b substituents are in adjacent positions in the

aromatic system, they may form a ring. Further, rings may be included in substituents which contain sufficient carbon atoms and heteroatoms to provide this possibility.

Preferred non-interfering substituents include hydrocarbyl groups of 1-6C, including saturated and unsaturated, linear or branched hydrocarbyl as well as hydrocarbyl groups containing ring systems; halo groups, alkoxy, hydroxy, amino, monoalkyl- and dialkylamino where the alkyl groups are 1-6C, CN, CF_3, and COOR.

Although the number of R^a and R^b substituents may typically be 0-4 or 0-5 depending on the available positions in the aromatic system, preferred embodiments include those wherein the number of R^a is 0, 1 or 2 and of R^b is 0, 1 or 2.

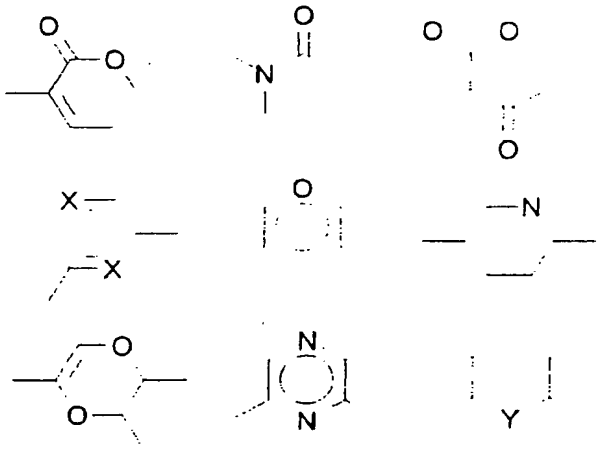
The linker group, L, may be a covalent bond or any group having a valence of at least two and covering a linear distance of from about 1.5 to about 15 Angstroms, including those that contain cyclic moieties, that meet this spatial requirement. Useful linkers are divided, by definition herein, into three general categories: (1) flexible non-conjugating linkers, (2) flexible conjugating linkers, and (3) constrained linkers. The preferred choice of linker will depend on the choices for Ar^1 and Ar^2. Not all of the linkers defined below are suitable for all Ar^1 and Ar^2 combinations.

As defined herein, *flexible non-conjugating* linkers are those that link only one position of Ar^1 to one position of Ar^2, and provide only a single covalent bond or a single chain between Ar^1 and Ar^2. The chain may contain branches, but may not contain π-bonds (except in the branches) or cyclic portions in the chain. The linker atoms in the chain itself rotate freely around single covalent bonds, and thus the linker has more than two degrees of freedom. Particularly useful flexible non-conjugating linkers, besides a covalent bond, are those of the formulae: -NR-, $-CR_2-$, -S-, or -O-, wherein R is H or alkyl (1-6C), more preferably H or lower alkyl (1-4C) and more preferably H. Also preferred are those of the formulae: -NRCO-, -CONR-, $-CR_2S-$, $-SCR_2-$, $-OCR_2-$, $-CR_2O-$, -NRNR-, $-CR_2CR_2-$, $-NRSO_2-$, $-SO_2NR-$, $-CR_2CO-$, $-COCR_2-$, and -NR-NR-CO-CR_2- and its complement $-CR_2$-CO-NR-NR-, including the isosteres thereof. Also preferred are those of the formulae: $-NR(CR_2)_2NR-$, $-O(CR_2)_2O-$, and $-S(CR_2)_2S-$, including the isosteres thereof. The optimum choice of linker within this group is dependent on the nature of Ar^1 and Ar^2.

Flexible conjugating linkers are those that link only one position of Ar^1 to one position of Ar^2, but incorporate at least one double or triple bond and/or one or more cyclic systems and thus have only two degrees of freedom. A flexible conjugating linker may

form a completely conjugated π-bond linking system between Ar^1 and Ar^2, thus providing for co-planarity of Ar^1 and Ar^2. Examples of useful flexible conjugating linkers include: -RC=CR-; -N=N-; -C≡C-; -RC=N-; -N=CR-; -NR-N=CR-; -NR-NR-CO-CR=CR-; and the like, where R is H or alkyl (1-6C); preferably H or lower alkyl (1-4C); and more preferably H.

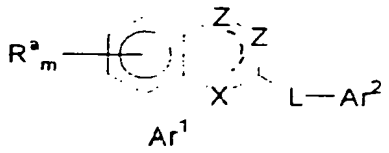
Constrained linkers are those that have more than one point of attachment to either or both Ar^1 and Ar^2 and, thus, generally allow for only one degree of freedom. Constrained linkers most frequently form fused 5- or 6-membered cyclic moieties with Ar^1 and/or Ar^2 where either Ar^1 or Ar^2 has at least one substituent appropriately positioned to form a second covalent bond with the linker, e.g., where Ar^2 is a phenyl group with a reactive, ortho-positioned substituent, or is derivatized to the linker directly at the ortho position. (Although the aromatic moieties should properly be referred to as phenylene or naphthylene in such cases, generally the term "phenyl" or "naphthyl" is used herein to include both monovalent and bivalent forms of these moieties.) Examples of particularly useful constrained linkers include



and the like, where X is O, N, S or CR, and Y is CR_2 or C=O.

Many of the compounds useful in the invention are commercially available and can be synthesized by art-known methods. Those compounds useful in the invention which are new compounds, can similarly be obtained by methods generally known in the art.

In one set of compounds of the inventions, Ar^1 is a substituted or unsubstituted aromatic system containing a six-membered heterocycle and the compounds useful in the invention have the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

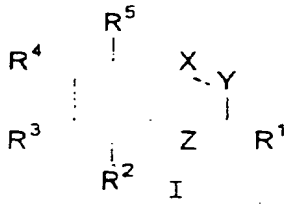
each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

A particularly preferred set of embodiments is of the formula:



I

in which:

R^1 is taken from the group: N=NAr, NR^6COAr, $CONR^6Ar$, CH_2OAr, CH_2NR^6Ar, where Ar is a six-membered (un)substituted aromatic ring. Allowable substituents on this aromatic ring include:

halogen, straight or branched chain lower alkyl, alkenyl, or alkynyl, optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, siloxy, acyloxy, straight or branched chain lower alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy, in which

R^6 is taken from the group: hydrogen, or straight or branched chain lower alkyl;

R^2 and R^5 are individually taken from the group: H, hydroxy, siloxy, acyloxy, halo, cyano, straight or branched chain lower alkyl, or straight or branched chain lower alkoxyl;

R^3 and R^4 are individually taken from the group: H, halogen, straight or branched chain lower alkyl, alkenyl, or alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, siloxy, acyloxy, straight or branched chain lower alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, and carboxy;

X and Y are either: NR^8 and N, respectively, in which case X and Y are singly bonded, or CR^9 and CR^{10}, respectively, in which case X and Y are doubly bonded, wherein

R^8 is either H or lower alkyl;

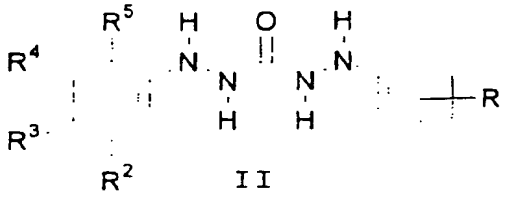
R^9 and R^{10} are individually taken from the group: H, halo, and lower alkyl;

Z is taken from the group: O, S, SO, and SO_2;

or salts thereof.

Compounds of the general structure I above can be prepared in a variety of ways, for example:

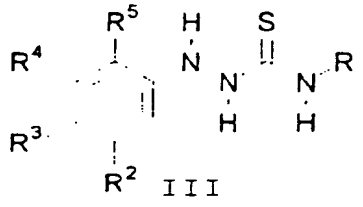
a) treating thiohydrazides of general structure II, or the corresponding thiohydrazones, in hot acetic acid in air,



II

; or

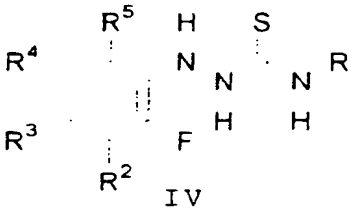
b) reacting compounds of the general structure III with bromine,



III

; or

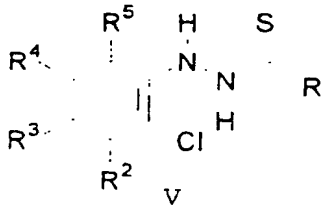
c) heating compounds of general structure IV in a protic solvent,



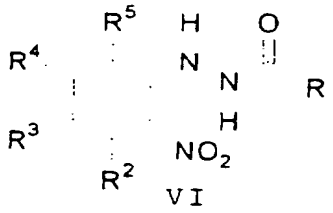
IV

; or

d) reacting compounds of the general structures V or VI with sodium hydride,



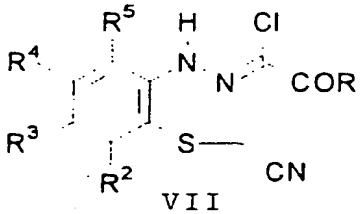
V



VI

; or

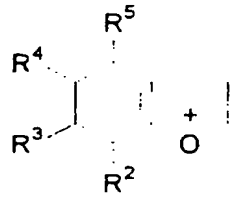
e) reacting compounds of the general structure VII with a base,



VII

; or

f) reacting pyrylium compounds of general structure VIII with an appropriate nucleophile,

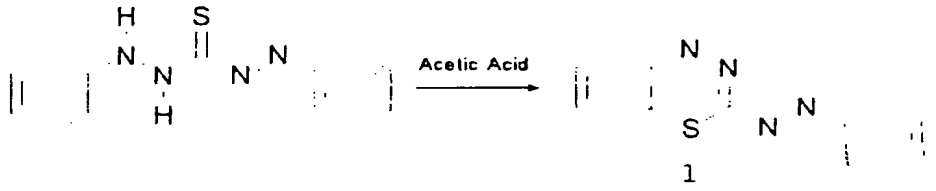


VIII

where R^2, R^3, R^4, R^5, are as defined above and R is taken from the group: Ar, NHAr, NHNHAr, COAr, carboalkoxy, alkoxy, NR^6COAr, CH_2OAr, and CH_2NR^6Ar, in which Ar and R^6 are as described above, followed, optionally, by conversion of any one or more of the groups, R, R^2, R^3, R^4, R^5 into new groups R, R^2, R^3, R^4, R^5 by deprotection, coupling, addition, substitution, or elimination; or by oxidation of the sulfur to sulfoxide or sulfone; and, if desired, by converting a compound of the general structure I into its salt or setting it free from its salt.

Example:

Diphenyl thiohydrazone is heated in refluxing acetic acid in air for 30 to 90 minutes to afford benzothiadiazene **1**.



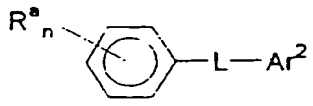
1

Specific representatives of compounds of the general structure I include:

3-phenylazo-1H-4,1,2-benzothiadizine

2-phenylazo-2H-benzopyran

Another group of compounds suitable for use in the methods of the invention are compounds of the formula:



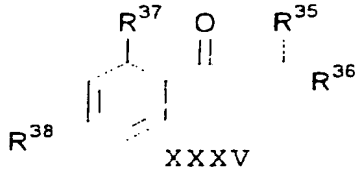
wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a flexible linker which does not contain nitrogen; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

Particularly preferred embodiments of this group of compounds are those of the formula:



XXXV

in which

R^{35} is taken from the group: H, hydroxy, alkoxyl, acyloxy, and silyloxy;

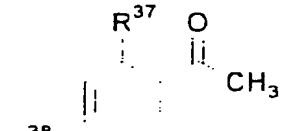
R^{36} is either Ar, or COAr, in which Ar is (un)substituted phenyl in which the allowed substituents are taken from the group: H, hydroxy, (un)substituted alkoxy, acyloxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, or (un)substituted alkynyl, carboxy, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl, and aryl;

R^{37} is taken from the group: H, hydroxy, alkoxy, halo, acyloxy, and siloxyl;

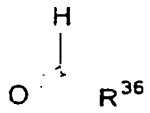
R^{38} is taken from the group: H, hydroxy, alkoxy, acyloxy, siloxy, (un)substituted alkoxy, acyloxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, and (un)substituted alkynyl,

or salts thereof. --

Compounds of general structure XXXV can be prepared by treating an acetophenone of general structure XXXVI with an appropriate aldehyde of general structure XXXVII under either basic or acidic conditions,

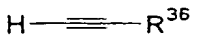


XXXVI

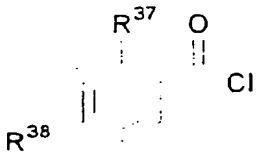


XXXVII

or by treating an appropriate alkyne of general structure XXXVIII with an acid halide of general structure XXXIX in the presence of a suitable catalyst, such as aluminum trichloride,

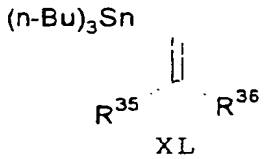


XXXVIII



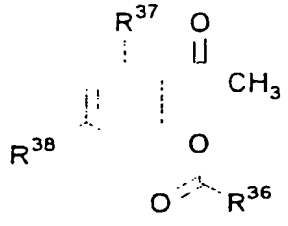
XXXIX

or by treating acid halides of the general structure XXXIX with (E)-1,2-bis(tri-n-butylstanyl)ethylene, or with a vinylstanane of general structure XL in the presence of a suitable catalyst, for example, a palladium catalyst.



XL

or by treating an acetophenone of general structure XLI with a strong base,



XLI

where R^{35}, R^{36}, R^{37}, and R^{38} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{35}, R^{36}, R^{37}, and R^{38} into new groups R^{35}, R^{36}, R^{37}, and R^{38} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XXXV into its salt or setting it free from its salt.

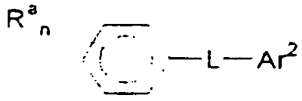
Specific representative compounds of general structure XXXV include:

2,4-dimethoxy-2'-hydroxychalcone

1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)propan-1,3-dione

1,4-dioxo-1,4-diphenylbut-2-ene

Still another group of compounds useful in the invention are those of the formula:



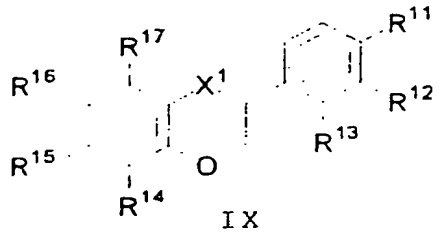
wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

Particularly preferred compounds in this group are those of formulas IX, XIV, and XX as follows:



IX

in which:

R^{11} and R^{12} are individually taken from the group:

H, hydroxy, C_{1-6} alkoxy, acetyloxy, and C_{1-12} (un)substituted alkyl;

R^{13}, R^{14} and R^{17} are individually taken from the group:

H, hydroxy, C_{1-6} straight or branched chain alkoxy, and acetyloxy;

R^{15} is taken from the group: Hydroxy, (un)substituted C_{1-12} alkoxy, C_{1-12} alkyl, (un)substituted alkenyl, and acetyloxy;

R^{16} is taken from the group: H, hydroxy, (un)substituted lower alkoxy, acetoxy, (un)substituted alkyl, and (un)substituted alkenyl;

where R^{11}, R^{12} may form a 5-7 member (un)substituted carbocycle or heterocycle;

where R^{15}, R^{16} may form a 5-7 member (un)substituted carbocyclic or heterocyclic ring;

X^1 is either carbonyl or CH_2;

and the dotted line may be a double bond,

in which permissible substituents on the above mentioned substituted groups include: Lower alkyl, lower alkoxyl, hydroxy, siloxy, halo, carboxyl, and aryl, with the following provisions:

if X^1 is carbonyl and

if R^{15} is hydroxy and if only one of R^{11}, R^{12}, or R^{13} is hydroxy, then at least one of R^{14}, R^{16}, and R^{17} must be other than H;

or if R^{15} is alkoxy, and if R^{11}, R^{12}, R^{13} together are H, then R^{17} can be neither H nor hydroxy;

or if R^{15} is (un)substituted alkoxy, and if R^{11}, R^{12}, and R^{13} together consist of only H, or H and one or two alkoxy, and R^{17} is H, then R^{14} must be other than H, Me or hydroxymethyl;

or if R^{15} is hydroxy or alkoxy, and if R^{11}, R^{12}, R^{13} together consist of only H, or H and one or two alkoxy, or H and only one or two alkyl, and R^{17} is C_{1-4} alkyl, then at least one of R^{14} and R^{16} must be other than H;

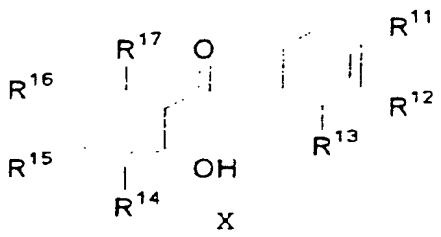
or if R^{15} is hydroxy and if R^{11}, R^{12}, R^{13}, R^{14}, and R^{16} all are H, R^{17} must be neither H nor hydroxy;

or if R^{15} is iso-propoxy, and if R^{11}, R^{12}, and R^{13} together consist of only H, or H and one or two hydroxys, then at least one of R^{14}, R^{16}, R^{17} must be other than H;

or if R^{15} is 1,5 di(lower) alkyl C_{5-10} alkyl, then at least one of R^{11}, R^{12}, R^{13}, R^{14}, R^{16}, and R^{17} must be other than H;

or salts thereof.

Compounds of the general structure shown above can be made by a process wherein ketones of the structure (X) shown below:



are reacted with an alkylorthoformate in the presence of a base, or

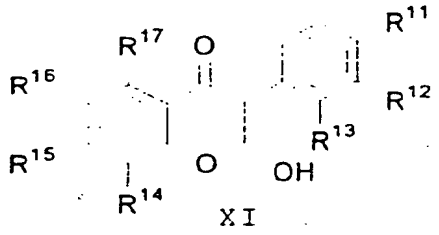
are reacted with an ethyloxalyl chloride in the presence of pyridine, followed by hydrolysis and decarboxylation, or

are reacted with an alkyl formate in the presence of an alkali metal, or

are reacted with an N,N-dialkyl formamide in the presence of phosphorous oxychloride, or

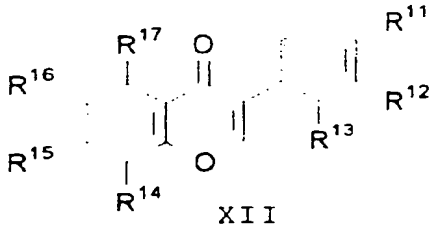
are reacted with a cyanide in the presence of hydrogen halide,

or by dehydrating 2-hydroxyisoflavanoids of the general structure (XI):



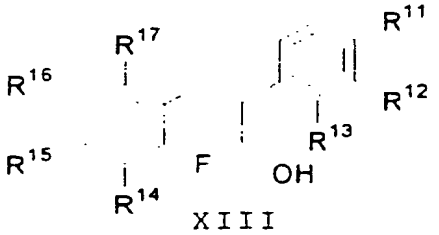
XI

or by subjecting compounds of the general structure XII to catalytic hydrogenation,



XII

or by treating compounds of the general structure XIII, available from alkylation of the corresponding phenylacetate with an appropriate benzylhalide, followed by reduction, with $(PF_6)_2Rh(EtC_5Me_4)(C_6H_6)$,



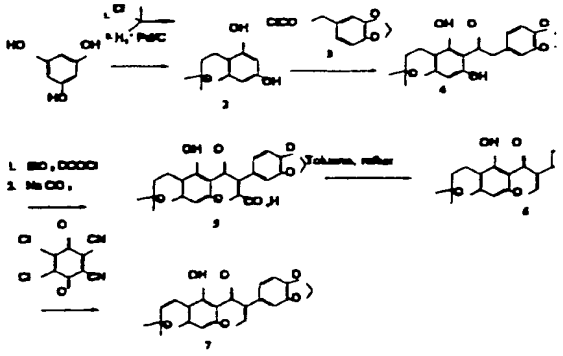
XIII

in which the groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} are as defined above, followed, optionally, by the conversion of any one or more of groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} into new groups R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, and R^{17} by deprotection,

dehydrogenation, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure IX into its salt or setting it free from its salt.

Example:

1,3,5-trihydroxybenzene is allowed to react with iso-pentynyl chloride, followed by catalytic hydrogenation, to give product **2**. The compound **2** is allowed to react with the acid chloride **3** to provide the ketone **4**. Ketone **4** is treated with ethyloxalyl chloride in pyridine at 0°C to afford an ester, which is hydrolyzed in aqueous acetone containing sodium carbonate to give the acid **5**. When heated in refluxing toluene, acid **5** undergoes decarboxylation to give compound **6**, which upon treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gives the isoflavanoid **7**.



Specific representatives of compounds of the general structure IX include:

tectorigenin

robustone

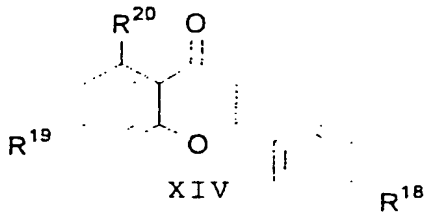
robustone methyl ether

7,2',4'-trihydroxyisoflavone

6,2',3'-trihydroxy-7,4'-dimethoxyisoflavan

8,4'-dimethoxy-7-hydroxisoflavone

Compounds of XIV have the structure:



XIV

in which:

R^{18} and R^{19} are individually taken from the group:

H, hydroxy, (un)substituted alkyl, (un)substituted alkoxy, COR^{21} carboxy, carboalkoxy, OR^{22}, carbamoyl optionally substituted at the nitrogen by lower chain alkyl or phenyl, acyloxy, halo, cyano, and azido.

R^{20} is taken from the group: H, hydroxy, halo, lower chain alkyl, acyloxy, and siloxy;

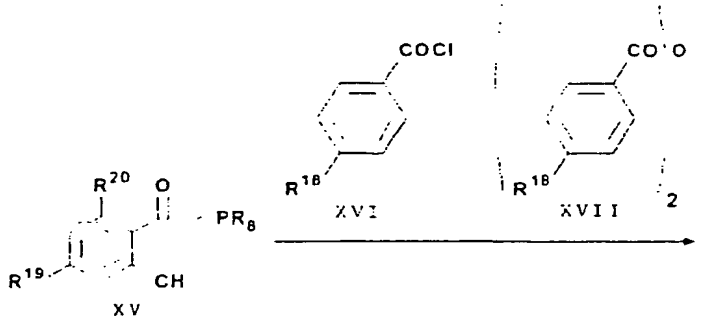
in which R^{21} is taken from the group: Alkyl, alkenyl,

alkynyl, aralkyl, (un)substituted phenyl, (un)substituted naphthyl, thienyl, furanyl, and pyridyl;

and R^{22} is comprised of a C_{5-6} carbohydrate moiety;

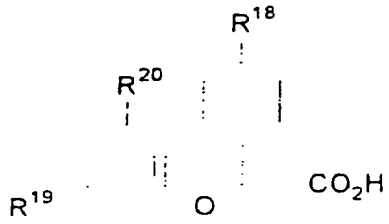
or salts thereof.

Compounds of general structure XIV can be prepared by reacting ylides of general structure XV with either acid chlorides of general structure XVI or acid anhydrides of general structure XVII:



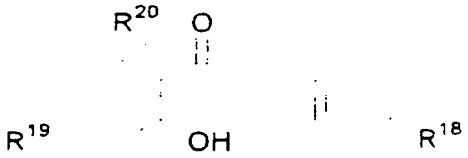
XV XVI XVII

or by treating acids of the general structure XVIII with polyphosphoric acid, trifluoracetic anhydride, or similar reagent,



XVIII

or by treating chalcones of general structure XIX with either base, or with base followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.



XIX

in which the groups R^{18}, R^{19}, R^{20} are as above, followed, optionally, by conversion of any one or more of the groups R^{18}, R^{19}, R^{20} into new groups R^{18}, R^{19}, R^{20} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of general structure XIV into its salt or setting it free from its salt.

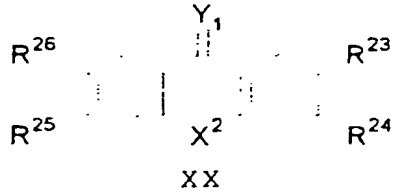
Specific representatives of compounds of the general structure XIV are:

5,4'-dimethyl-7-acetylflavone

7-benzoyloxyflavanone

apiin acetate

Compounds of structure XX are of the formula:



XX

where

R^{23}, R^{24}, R^{25}, R^{26} are individually taken from the group:

H, hydroxy, (un)substituted alkoxy, siloxy, (un)substituted alkyl, (un)substituted alkenyl, halo, carboxyl, and acyloxy, and where R^{23} and R^{24}, and likewise R^{25} and R^{26}, can together equal a 5-7 member (un)substituted carbocyclic or heterocyclic ring, and where substituents on the above mentioned optionally substituted groups may include lower chain alkyl, lower chain alkoxy, hydroxy, siloxy, acyloxy, halo, benzoyl, carboxy, carboalkoxy, and carbamoyl optionally substituted at nitrogen with lower chain alkyl, phenyl, thienyl, furyl, or pyridinyl;

Y^1 is taken from the group: O, $-OCH_2CH_2O-$, $-OCH_2CH_2S-$, $-OCH_2CH_2CH_2O-$, $-SCH_2CH_2CH_2S-$, and $-SCH_2CH_2S-$;

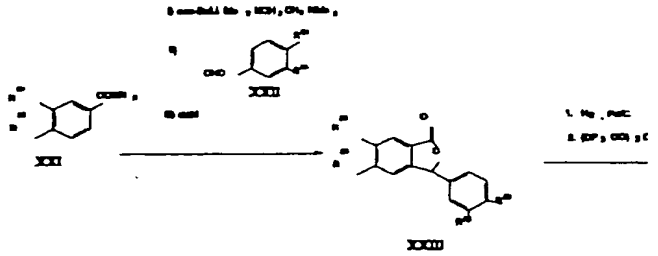
X^2 is taken from the group: CH_2, O, and S;

with the following provisions:

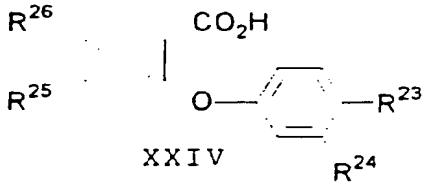
if X and Y are O and R^{24} or R^{25} are either both alkoxy, or alkoxy and alkyl, irrespective of order, then at least one of R^{23} and R^{26} must be other than H,

or salts thereof.

Compounds of the general structure XX can be prepared by reacting amides of general structure XXI with *sec*-butyl lithium and tetramethylethylenediamine in THF, followed by addition of benzaldehydes of general structure XXII, and the addition of acid. The resulting lactones of general structure XXIII can be reduced by catalytic hydrogenation or treatment with activated zinc in acid, followed by dehydration with trifluoracetic anhydride.



or, by treating diaryl ethers of general structure XXIV with sulfuric acid, alumium trichloride, trifluoracetic anhydride, or similar reagent,



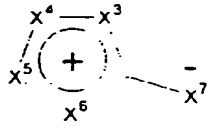
XXIV

where R^{23}, R^{24}, R^{25}, R^{26} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{23}, R^{24}, R^{25}, R^{26} into new groups R^{23}, R^{24}, R^{25}, R^{26} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XX into its salt or setting it free from its salt.

Specific representatives of compounds of the general structure XX include:

3-isopropoxyanthrone

Another group that is useful in the invention are of the formula:



XXV

in which:

X^3 is NR^{27}, X^4 is CR^{30}, X^5 is O, X^6 is CR^{31}, X^7 is O^-;

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{31}, X^6 is CR^{28}, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is NR^{28}, X^6 is CR^{31}, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{28} or N, X^5 is NR^{30}, X^6 is CR^{29}, X^7 is NR^{32};

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{28}, X^6 is CR^{29}, X^7 is NR^{32};

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is S, X^6 is CR^{31}, X^7 is NR^{32};

or X^3 is NR^{30}, X^4 is CR^{27}, X^5 is S, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is CR^{31}, X^7 is O^- or S^-;

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{27}, X^5 is NR^{30}, X^6 is CR^{28}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is S, X^6 is CR^{27}, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is S, X^6 is CR^{31}, X^7 is O^-;

or X^3 is NR^{30}, X^4 is CR^{27} or N, X^5 is NR^{31}, X^6 is N, X^7 is O^- or S^-;

or X^3 is NR^{27}, X^4 is CR^{30}, X^5 is NR^{28}, X^4 is N, X^7 is NR^{32} or CZ^2Z^3;

or X^3 is NR^{27}, X^4 is CR^{28} or N, X^5 is NR^{30}, X^4 is N, X^7 is NR^{32} or CZ^2Z^3;

or X^3 is NR^{30}, X^4 is N, X^5 is S, X^6 is CR^{31}, X^7 is O^-;

or X^3 is S, X^4 is CR^{27}, X^5 is NR^{30}, X^6 is N, X^7 is NR^{32};

or X^3 is S, X^4 is CR^{30}, X^5 is NR^{27}, X^6 is N, X^7 is NR^{32};

or X^3 is O or S, X^4 is N, X^5 is NR^{30}, X^6 is N, X^7 is NR^{32};

in which

R^{27}, R^{28} and R^{29} are individually straight or branched chain lower alkyl;

R^{30} and R^{31} are individually taken from the group:

hydrogen, straight or branched chain (un)substituted alkyl, (un)substituted aromatic, in which the substituents may include: Halogen, straight or branched chain lower alkyl, alkenyl, alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, straight or branched chain alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy;

R^{32} is taken from the group:

Ar, COAr, COR^{33}, where Ar is a six-membered (un)substituted aromatic ring, in which substituents on this ring may include: Halogen, straight or branched chain lower alkyl, alkenyl, alkynyl optionally substituted by a six-membered aromatic, cyclic alkyl, or cyclic alkenyl ring, hydroxyl, straight or branched chain alkoxyl, benzoyl, carboalkoxy, carbamoyl optionally substituted at nitrogen by lower chain alkyl or phenyl, or carboxy;

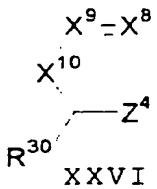
R^{33} is taken from the group: Hydrogen, and straight or branched chain alkyl;

Z^2 and Z^3 are individually taken from the group: CN and CO_2R^{34};

R^{34} is taken from the group: Hydrogen, straight or branched chain alkyl, and (un)substituted aromatic;

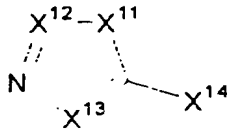
or salts thereof.

Compounds of general structure XXV above can be prepared by treating compounds of general structure XXVI, where X^8 is NR^{30} or S, X^9 is CR^{30} or N, X^{10} is NR^{30} or S, Z^4 is CO_2H, CO_2R^{30} or CN, with acid chlorides or anhydrides,



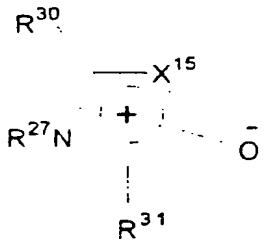
XXVI

or by reacting compounds of general structure XXVII, where X^{11} is NR^{30} or S, X^{12} is N or CR^{30}, X^{13} is halogen, SMe, or OEt, with amines, sulfides or enolates.



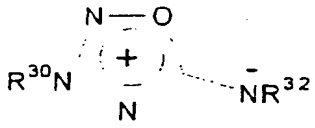
XXVII

or by reacting compounds of general structure XXVIII, where X^{15} is O or S with isocyanates, isothiocyanates, or carbon disulfide.



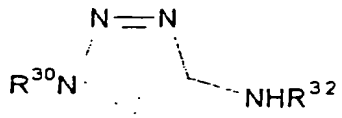
XXVIII

or by reacting compounds of general structure XXIX with sodium hydroxide.



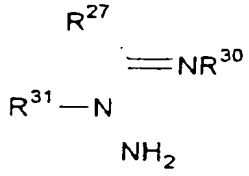
XXIX

or by reacting compounds of general structure XXX with alkyl tosylates, aryl tosylates or alkyl halides.



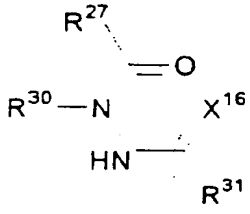
XXX

or by reacting compounds of general structure XXXI with aryl isocyanide dichlorides, phosgene, thiophosgene, or 3,3-bis(methylthio)acrylonitriles,



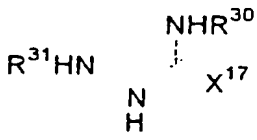
XXXI

or by reacting compounds of general structure XXXII, where X^{16} is O, S, or NH, with sodium ethoxide or HCl in the presence of acid chlorides or HCl in the presence of acid anhydrides,



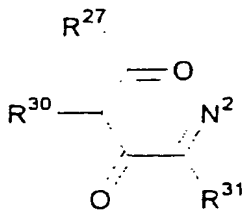
XXXII

or by reacting compounds of general structure XXXIII, where X^{17} is NH or S, with acid chlorides, acid anhydrides, or HONO,



XXXIII

or by reacting compounds of general structure XXXIV with $Cu(acac)_2$,



XXXIV

where R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} are as defined above, followed, optionally, by conversion of any one or more of the groups R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} into new groups R^{22}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} by deprotection, coupling, addition, substitution, or elimination, and, if desired, by converting a compound of the general structure XXV into its salt or setting it free from its salt.

Specific representatives of compounds of the general structure XXV include:

3-(4-chlorophenyl)-1,2,3,4-oxatriazolium-5-(4-chlorophenyl)aminide

1,3-di(4-methylphenyl)-1,2,3,4-tetrazolium-5-oxide.

The following examples are intended to illustrate, but not to limit, the invention.

Example 1

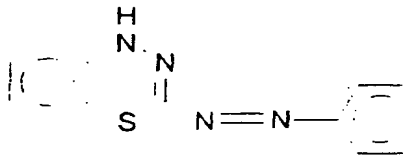
Compound 59-0008 was synthesized according to the procedure of McDonald, W. S., *et al. Chem Comm* (1969) 392-393; Irving, H. N. N. H. *et al. Anal Chim Acta* (1970) 49:261-266. Briefly, 10.0 g of dithizone was taken up in 100 ml EtOH and 50 ml AcOH and heated at reflux for 18 h. After cooling, this was diluted first with 100 ml water and then with 50 ml 1N NaOH. This was then further neutralized by the addition of 6 N NaOH to bring the pH to 5.0. This deep purple mixture was then concentrated on a rotavapor to remove organics. Once the liquid had lost all of its purple color, this was filtered to collect the dark precipitate. Purification by flash chromatography (4.5 x 25.7 cm; EtAc/Hep. (1:4); R_f 0.22) followed by recrystalization from EtOH gave 2.15 g (25% yield) of dark

purple crystals, mp=184-185 °C. ^{1}H NMR ($CDCl_3$) 7.90 (d of d, J_1=7.7, J_2=2.2, 2H), 7.64 (hump, 1H), 7.49 (m, 3H), 7.02 (m, 1H), 6.91 (m, 2H), 6.55 (d, J=8.1, 1H). MS (EI) 254 (47, M+), 105 (26), 77 [100], 51 (27). HRMS (EI, M+) 254.0626 (calcd 254.0626182). Anal. Calcd for $C_{13}H_{10}N_4S$: C, 61.40; H, 3.96; N, 22.03. Found: C, 61.40; H, 4.20; N, 22.06.

Example 2

High Throughput Screening

Several thousand compounds were tested in the assay system set forth in U.S. Serial No. 08/458,434, filed 2 June 1995, and incorporated herein by reference. The standard positive control was a compound of the invention, 59-0008 (also denoted "OS8"), which is of the formula:



"OS8"

In more detail, the 2T3-BMP-2-LUC cells, a stably transformed osteoblast cell line described in Ghosh-Choudhury *et al. Endocrinology* (1996) 137.331-39, referenced above, was employed. The cells were cultured using α-MEM, 10% FCS with 1% penicillin/streptomycin and 1% glutamine ("plating medium"), and were split 1:5 once per week. For the assay, the cells were resuspended in a plating medium containing 4% FCS, plated in microtiter plates at a concentration of 5 x 10^3 cells (in 50 µl)/well, and incubated for 24 hours at 37°C in 5% CO_2. To initiate the assay, 50 µl of the test compound or the control in DMSO was added at 2X concentration to each well, so that the final volume was 100 µl. The final serum concentration was 2% FCS, and the final DMSO concentration was 1%. Compound 59-0008 (10 µM) was used as a positive control.

The treated cells were incubated for 24 hours at 37°C and 5% CO_2. The medium was then removed, and the cells were rinsed three times with PBS. After removal of excess PBS, 25 µl of 1X cell culture lysing reagent (Promega #E153A) was added to each well and incubated for at least ten minutes. Optionally, the plates/samples could be frozen at

this point. To each well was added 50 µl of luciferase substrate (Promega #E152A; 10 ml Promega luciferase assay buffer per 7 mg Promega luciferase assay substrate). Luminescence was measured on an automated 96-well luminometer, and was expressed as either picograms of luciferase activity per well or as picograms of luciferase activity per microgram of protein.

In this assay, compound 59-0008 (3-phenylazo-1H-4,1,2-benzothiadiazine) exhibited a pattern of reactivity, as shown in Figure 1. The activity for compound 59-0008 was maximal at a concentration of approximately 3-10 µM and, more particularly, at about 3 µM, and thus provided a response of approximately 175 light emission units. Accordingly, other tested compounds were evaluated at various concentrations, and these results were compared to the results obtained for 59-0008 at 10 µM (which value was normalized to 100). For instance, any tested compound in Figure 2 and Figure 3 that showed greater activity than 10 µM of 59-0008 would result in a value over 100.

As shown in Figure 2 (39 sheets) and Figure 3 (10 sheets), several compounds were found to be particularly effective.

Example 3

In vivo Calvarial Bone Growth Data

Compound 59-0008 was assayed *in vivo* according to the procedure described previously (see "*In vivo* Assay of Effects of Compounds on Murine Calvarial Bone Growth", *supra*). As compared to a vehicle control, compound 59-0008 induced a 4-fold increase in width of new calvarial bone.

Example 4

Chondrogenic Activity

Compounds 59-008, 59-0102 and 50-0197 were assayed for effects on the differentiation of cartilage cells, as compared to the action of recombinant human BMP-2. Briefly, a mouse clonal chondrogenic cell line, TMC-23, was isolated and cloned from costal cartilage of transgenic mice containing the BMP-2 gene control region driving SV-40 large T-antigen, generated as described in Ghosh-Choudhury *et al Endocrinology* 137:331-39, 1996. These cells were cultured in DMEM/10% FCS, and were shown to

express T-antigen, and also to produce aggrecan (toluidine blue staining at pH 1.0) and Type-II collagen (immunostaining) by 7 days after confluence.

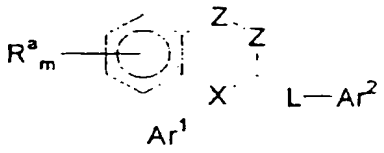
For measurement of alkaline phosphatase (ALP) activity, the technique of LF Bonewald *et al. J Biol Chem* (1992) 267:8943-49, was employed. Briefly, TMC-23 cells were plated in 96 well microtiter plates in DMEM containing 10% FCS at 4 x 10^3 cells/well. Two days after plating, the cells were confluent and the medium was replaced with fresh medium containing 10% FCS and different concentrations of compounds or recombinant BMP-2. After an additional 2 or 5 days incubation, the plates were washed twice with PBS, and then lysing solution (0.05% Triton X-100) was added (100 µl/well). The cells were lysed by three freeze-thaw cycles of -70°C (30 min), followed by 37°C (30 min with shaking). Twenty microliters of cell lysates were assayed with 80 µl of 5 mM p-nitrophenol phosphate in 1.5 M 2-amino-2-methyl-propanol buffer, pH 10.3 (Sigma ALP kit, Sigma Chemical Co., St. Louis, MO) for 10 min at 37°C. The reaction was stopped by the addition of 100 µl of 0.5 M NaOH. The spectrophotometric absorbance at 405 nm was compared to that of p-nitrophenol standards to estimate ALP activity in the samples. The protein content of the cell lysates was determined by the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Specific activity was calculated using these two parameters.

At day 2, compounds 59-0008 (10^{-9} M), 59-0102 (10^{-7} M) and 59-0197 (10^{-9} M) increased ALP levels approximately 3-, 2- and 2.5-fold, respectively, as compared to the vehicle control. Recombinant BMP2 at 100, 50 or 10 ng/ml induced ALP levels approximately 10-, 4- or 1.5-fold, respectively, as compared to the vehicle control.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

1. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

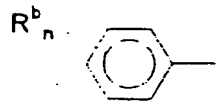
L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

2. The method of claim 1 wherein L is a flexible conjugated linker.

3. The method of claim 1 wherein L is selected from the group consisting of a covalent bond, -N=N-, -RC=CR-, -RC=N-, -N=CR-, -NRCO-, -CONR-, $-CR_2O-$, and $-CR_2NR-$ where each R is independently H or alkyl (1-6C).

4. The method of claim 1 wherein Ar^2 is



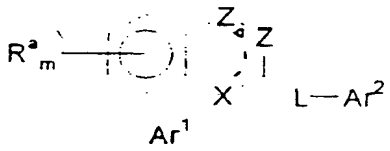
where R^b is a non-interfering substituent and n is an integer from 0 to 5.

5. The method of claim 4 wherein Ar^2 is unsubstituted phenyl.

6. The method of claim 1 wherein said compound is 59-0008.

7. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

m is an integer of 0-4;

each dotted line represents an optional π-bond;

each Z is independently N, NR, O, S, CR or CR_2, where each R is independently H or alkyl (1-6C);

X is O, S, SO or SO_2;

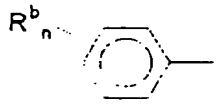
L is a flexible linker; and

Ar^2 is a substituted or unsubstituted 6-membered aromatic ring.

8. The composition of claim 7 wherein L is a flexible conjugated linker.

9. The composition of claim 7 wherein L is selected from the group consisting of a covalent bond, -N=N-, -RC=CR-, -RC=N-, -N=CR-, -NRCO-, -CONR-, $-CR_2O$, and $-CR_2NR$- where each R is independently H or alkyl (1-6C).

10. The composition of claim 7 wherein Ar^2 is

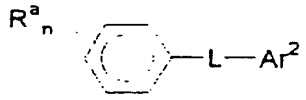


where R^b is a non-interfering substituent and n is an integer from 0 to 5.

11. The composition of claim 7 wherein Ar^2 is unsubstituted phenyl.

12. The composition of claim 7 wherein said compound is 59-0008.

13. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;
n is an integer of 0 and 5;
L is a flexible linker which does not contain nitrogen; and
Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

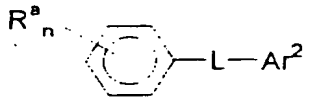
14. The method of claim 13 wherein R^a is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

15. The method of claim 13 wherein Ar^2 is substituted or unsubstituted phenyl.

16. The method of claim 13 wherein Ar^1 and Ar^2 are different.

17. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a flexible linker which does not contain nitrogen; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

18. The composition of claim 17 wherein R^a is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

19. The composition of claim 17 wherein Ar^2 is substituted or unsubstituted phenyl.

20. The composition of claim 17 wherein Ar^1 and Ar^2 are different.

21. A method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption, which method comprises administering to a vertebrate subject in need of such treatment an effective amount of a compound of the formula:

wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

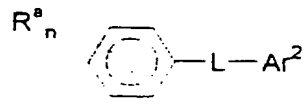
Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

22. The method of claim 21 wherein R^1 is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

23. The method of claim 21 wherein Ar^2 is substituted or unsubstituted phenyl.

24. A pharmaceutical composition for use in a method to treat a condition in a vertebrate animal characterized by a deficiency in, or need for, bone growth replacement and/or an undesirable level of bone resorption,

which composition comprises a pharmaceutically acceptable excipient and an effective amount of a compound of the formula:



wherein R^a is a non-interfering substituent;

n is an integer of 0 and 5;

L is a constrained linker; and

Ar^2 is a substituted or unsubstituted phenyl or a substituted or unsubstituted naphthyl.

25. The composition of claim 24 wherein R^1 is $-NR_2$ or -COOR, where R is H or alkyl (1-6C).

26. The composition of claim 24 wherein Ar^2 is substituted or unsubstituted phenyl.

27. The method of any of claims 1, 13 or 21 wherein said condition is osteoporosis, bone fracture or deficiency, primary or secondary hyperparathyroidism, periodontal disease or defect, metastatic bone disease, osteolytic bone disease, post-plastic surgery, post-prosthetic joint surgery, or post-dental implantation.

28. The method of any of claims 1, 13 or 21 which further comprises administering to said subject one or more agents that promote bone growth or that inhibit bone resorption.

29. The method of claim 28 wherein said agents are selected from the group consisting of bone morphogenetic factors, anti-resorptive agents, osteogenic factors, cartilage-derived morphogenic proteins, growth hormones, and differentiating factors.

2TJL-C9 CELLS		10/1/96					
5x 10^5 Cells/well							
	uM	READ 1	READ 2	AVERAGE	INDUCTION	AVE-BASAL	% MAX
OS-8	100.000	0.21	0.22	0.22	0.18	-0.99	-17.80
	31.250	3.98	4.44	4.20	3.49	3.00	54.26
	9.766	6.99	6.46	6.72	5.59	5.52	100.00
	3.052	4.62	4.88	4.75	3.95	3.55	64.22
	0.954	3.13	3.16	3.14	2.61	1.94	35.12
	0.298	2.75	2.59	2.67	2.22	1.47	26.58
	0.093	2.10	2.04	2.07	1.72	0.87	15.77
	0.029	1.56	1.71	1.63	1.36	0.43	7.80
	0.0091	1.45	1.42	1.44	1.19	0.23	4.21
	0.0028	1.28	1.37	1.33	1.10	0.12	2.25
	0.0000	1.32	1.30	1.31			
	0.0000	1.20	1.00	1.10			
	AVERAGE BASAL			1.20			

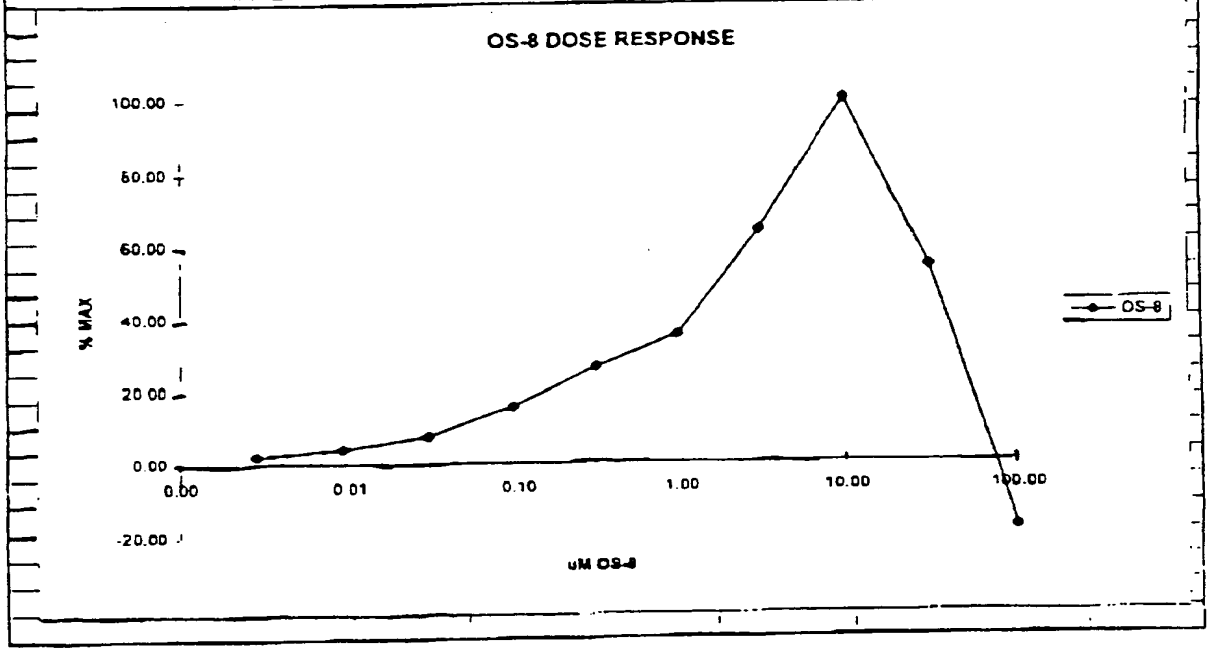


Figure 1

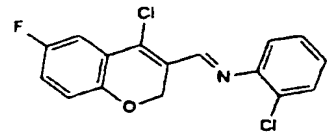
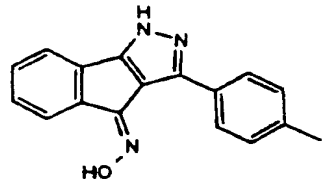
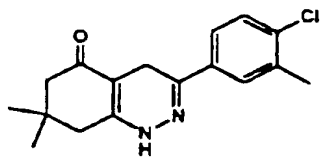
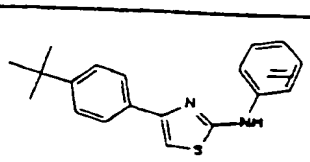
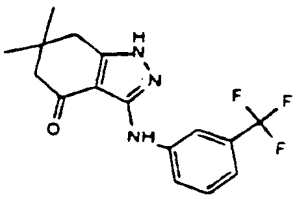
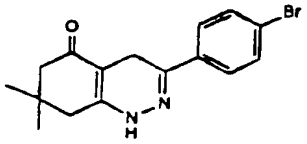
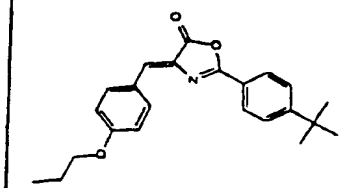
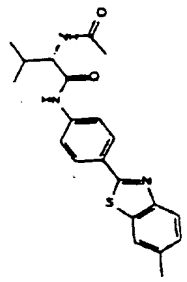
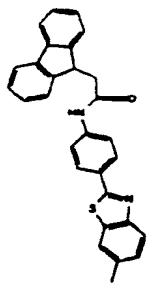
	21.433		69.74
233.289	4.287		31.59
	0.857		39.70
	0.171		18.29
 92-6353			
92-6353	155.199	uM	
	31.040		204.14
322.166	15.520		154.94
	3.104		28.09
	1.552		
	0.310		3.53
 92-8007			
92-8007	181.613	uM	-16.65
	36.323		58.65
275.311	18.161		142.33
	3.632		45.65
	1.816		
	0.363		4.47
 92-8215			
92-8215	165.123	uM	32.90
	33.025		151.08
302.805	16.512		132.29
	3.302		59.90
	1.651		
	0.330		23.34

Figure 3

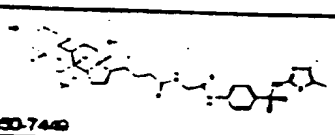
				
92-8258				
92-8258		162.102	uM	-16.65
		32.420		157.44
	308.447	16.210		101.04
		3.242		39.02
		1.621		
		0.324		12.78
				
92-8362				
92-8362		154.647	uM	138.79
		30.929		137.00
	323.318	15.465		85.02
		3.093		17.34
		1.546		
		0.309		0.41
				
92-8372				
92-8372		150.045	uM	63.76
		30.009		134.71
	333.234	15.004		92.08
		3.001		31.35
		1.500		
		0.300		13.20
				
92-8183				



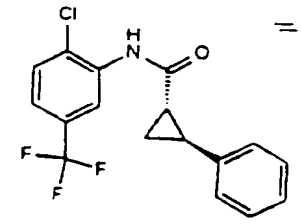
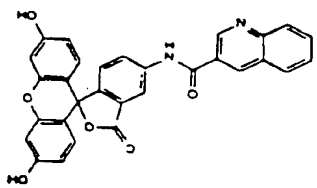
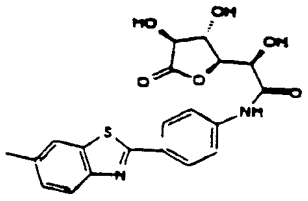
850-7377			
850-7377		131.062 uM	-50.32
		13.106	68.27
	381.498	2.621	116.81
		0.524	61.26
		0.105	35.86

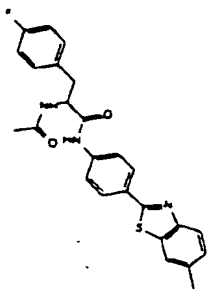
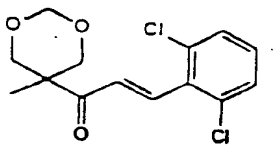
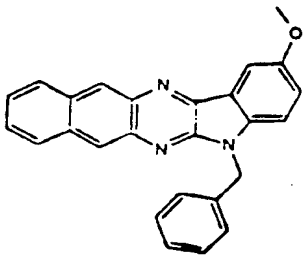


850-7413			
850-7413		111.964 uM	-40.44
		11.196	-2.55
	446.572	2.239	157.01
		0.448	78.73
		0.090	23.91

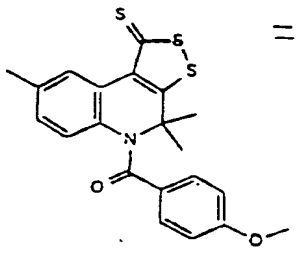


850-7449			
850-7449		89.938 uM	-42.42
		6.994	73.79
	714.823	1.399	112.16
		0.280	75.24
		0.056	26.36

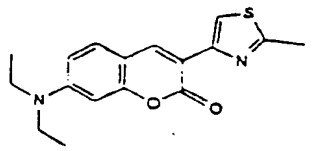
 850-9287			
850-9287		147.170 uM	
		14.717	-15.82
	339.744	2.943	15.82
		0.589	130.71
		0.118	91.11
			69.05
 850-9356			
850-9356		99.506 uM	
		9.951	-24.650
	502.482	1.990	83.140
		0.398	188.810
		0.080	45.470
			9.740
 850-9467			
850-9467		120.846 uM	
		12.085	-19.800
	414.436	2.413	112.980
		0.483	122.730
		0.097	43.520
			33.140

			
650-9576			
650-9576		111.724	uM
		11.172	
	447.532	2.234	
		0.447	
		0.089	
			
695-0262			
695-0262		166.019	uM
		33.204	
	301.189	16.602	
		3.320	
		0.332	
			
695-0268			
695-0268		128.363	uM
		25.677	
	369.458	12.838	
		2.568	
		0.257	

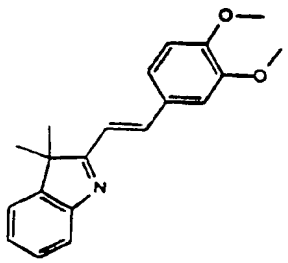
-27.430
90.560
101.810
44.900
19.930
-19.16
-12.60
146.26
-2.23
-3.07
-18.87
40.25
169.96
195.29
14.02



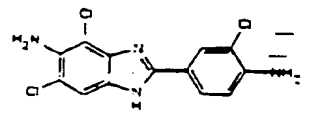
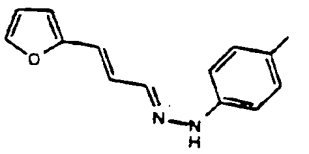
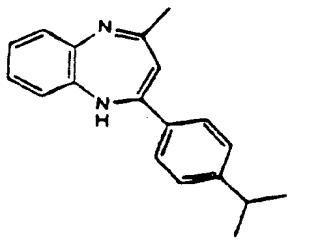
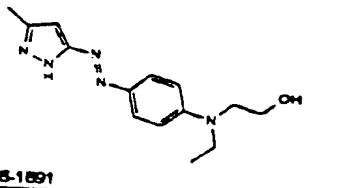
895-0594			
895-0594			
	120.898	uM	-21.83
	12.090		25.89
413.58	2.418		122.10
	0.484		75.32
	0.097		39.42

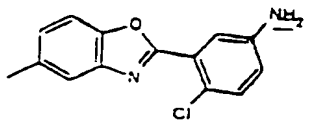
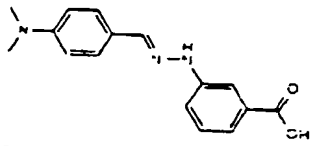
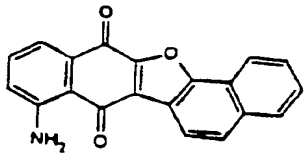
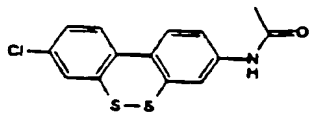


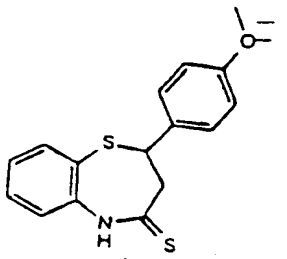
895-0657			
895-0657			
	159.028	uM	-30.48
	15.903		146.74
314.407	3.181		74.54
	0.636		25.82
	0.127		3.66



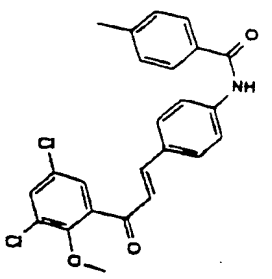
895-0664			
895-0664			
	162.655	uM	-31.08
	16.266		325.06
307.393	3.253		67.51
	0.651		40.39
	0.130		16.03

			
895-1161			
895-1161		152.625	uM
		15.263	
	327.602	3.053	
		0.611	
		0.122	
			-5.51
			109.31
			58.06
			29.49
			24.71
			
895-1420			
895-1420		220.965	uM
		22.097	
	228.279	4.419	
		0.884	
		0.177	
			-19.47
			110.90
			49.94
			33.65
			20.06
			
895-1679			
895-1679		180.910	uM
		18.091	
	276.383	3.618	
		0.724	
		0.145	
			-30.36
			111.72
			102.83
			18.01
			0.44
			
895-1691			
895-1691		182.922	uM
		18.292	
	273.34	3.658	
			-16.29
			50.84
			105.70

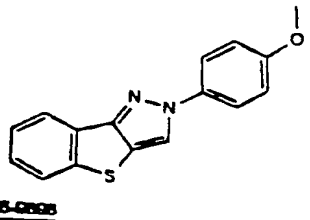
			
895-3646			
895-3846		193.267	uM
		9.327	
	258.708	3.865	
		0.773	
		0.155	
			-21.41
			13.40
			114.46
			52.12
			38.29
			
895-4642			
895-4642		176.473	uM
		17.647	
	283.331	3.529	
		0.706	
		0.141	
			6.97
			283.99
			447.51
			304.66
			100.45
			
895-4843			
895-4843		159.561	uM
		15.956	
	313.312	3.192	
		0.638	
		0.128	
			-17.18
			24.54
			100.12
			60.37
			27.85
			
895-5185			
895-5185		162.433	uM
		16.243	
	307.821	3.249	
		0.650	
		0.130	
			-6.47
			213.42
			107.83
			46.75
			18.27



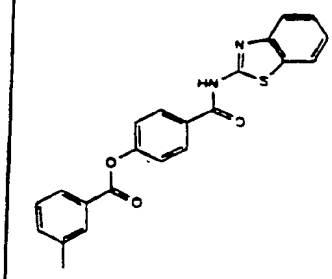
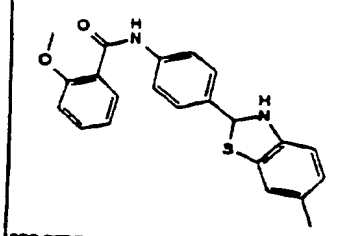
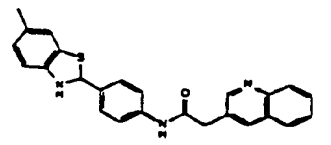
895-8662			
895-8662			
		185.876	uM
		16.888	
	301.43	3.318	
		0.884	
		0.133	
			54.72
			159.21
			113.97
			41.98
			36.28



895-8663			
895-8663			
		113.552	uM
		11.355	
	440.326	2.271	
		0.454	
		0.091	
			-20.87
			201.56
			12.95
			0.62
			-0.69



895-8668			
895-8668			
		178.349	uM
		17.835	
	280.349	3.567	
		0.713	
		0.143	
			-29.16
			0.62
			182.84
			118.55
			42.75

464.9791	2.151	188.84
—	0.430	108.12
—	0.096	37.18
		
808-0350		
808-0350	128.718 uM	-16.80
	12.872	87.23
388.445	2.574	210.25
	0.515	73.36
	0.103	28.25
		
808-0535		
808-0535	132.810 uM	-10.41
	13.281	73.84
378.478	2.656	199.80
	0.531	102.12
	0.106	35.72
		
808-0554		
808-0554	121.490 uM	-16.32
	12.150	105.48
411.527	2.430	115.43
	0.486	53.88
	0.097	27.03

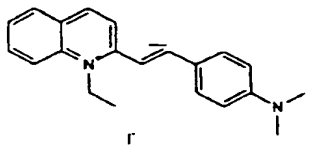
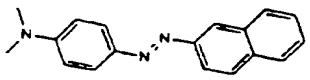
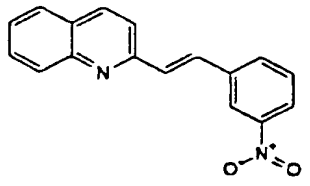
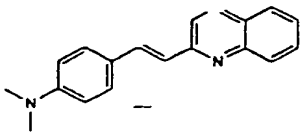
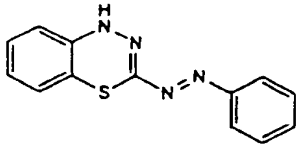
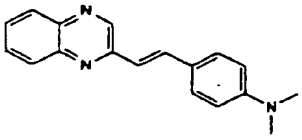
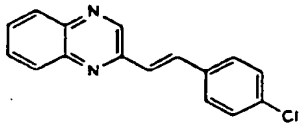
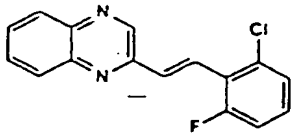
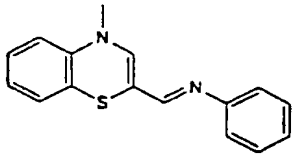
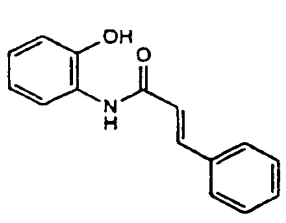
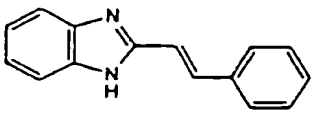
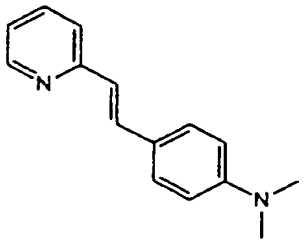
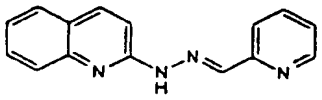
NNC#	MOL.WEIGHT	Concentration		% Response
				
50-0194	430.33			
50-0194		100.00	uM	-19.190
		31.25	uM	32.450
		9.77	uM	-14.240
		3.05	uM	-11.330
		953.67	nM	-12.790
		298.02	nM	-13.450
		93.13	nM	-12.290
		29.10	nM	-9.440
		9.09	nM	-6.450
		2.84	nM	-8.130
		888.18	pM	-3.320
				
50-0195	275.36			
50-0195		100.00	uM	-4.630
		31.25	uM	16.790
		9.77	uM	62.830
		3.05	uM	102.720
		953.67	nM	60.860
		298.02	nM	32.450
		93.13	nM	19.340
		29.10	nM	17.220
		9.09	nM	5.640
		2.84	nM	4.840
		888.18	pM	5.640
				
50-0196	276.30			
50-0196		100.00	uM	-16.210
		31.25	uM	-8.560
		9.77	uM	11.620
		3.05	uM	27.790
		953.67	nM	18.380
		298.02	nM	5.230
		93.13	nM	12.420
		29.10	nM	12.630
		9.09	nM	6.590
		2.84	nM	7.970
		888.18	pM	5.060

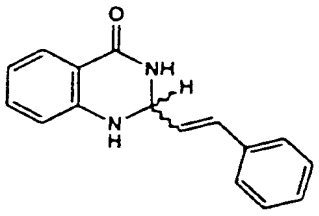
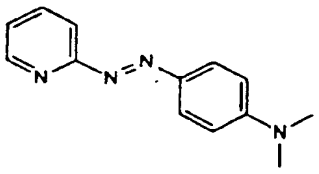
Figure 2

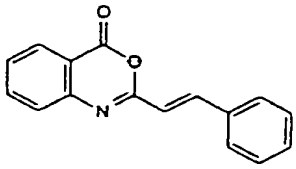
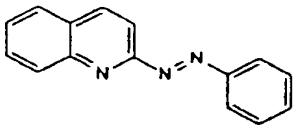
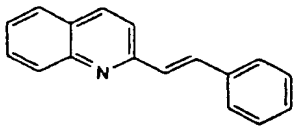
			
50-0197	274.37		
50-0197		100.00 uM	-18.250
		31.25 uM	-14.980
		9.77 uM	4.040
		3.05 uM	93.790
		953.67 nM	205.530
		298.02 nM	242.920
		93.13 nM	195.890
		29.10 nM	115.320
		9.09 nM	85.630
		2.84 nM	54.380
		888.18 pM	33.180
			
59-0008	254.32		
			
59-0019	59-0019		
59-0019		100.00 uM	-22.240
		31.25 uM	-22.870
		9.77 uM	-17.470
		3.05 uM	74.490
		953.67 nM	198.080
		298.02 nM	258.340
		93.13 nM	225.350
		29.10 nM	75.220
		9.09 nM	24.030
		2.84 nM	34.480
		888.18 pM	-3.740
			
59-0020	266.73		
59-0020		100.00 uM	-16.510
		31.25 uM	-16.040
		9.77 uM	-0.270
		3.05 uM	96.490
		953.67 nM	153.320
		298.02 nM	110.240
		93.13 nM	60.030

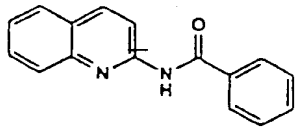
		29.10 nM	37.870
		9.09 nM	24.820
		2.84 nM	20.500
		888.18 pM	13.310

			
59-0021	284.72		
59-0021		100.00 uM	-16.310
		31.25 uM	-12.850
		9.77 uM	84.130
		3.05 uM	89.940
		953.67 nM	65.750
		298.02 nM	33.940
		93.13 nM	22.660
		29.10 nM	25.020
		9.09 nM	13.910
		2.84 nM	33.270
		888.18 pM	15.500
			
59-0022	268.37		
59-0022		100.00 uM	7.250
		31.25 uM	-2.070
		9.77 uM	-0.270
		3.05 uM	4.390
		953.67 nM	3.060
		298.02 nM	-1.800
		93.13 nM	-0.200
		29.10 nM	-3.270
		9.09 nM	1.130
		2.84 nM	2.590
		888.18 pM	2.460
			
59-0023	239.28		
59-0023		100.00 uM	-12.720
		31.25 uM	33.140
		9.77 uM	56.500
		3.05 uM	29.550
		953.67 nM	25.360
		298.02 nM	15.700
		93.13 nM	7.380
		29.10 nM	9.710
		9.09 nM	1.000
		2.84 nM	4.520
		888.18 pM	-0.010

 59-0024	220.28				
 59-0025	224.31				
59-0025		100.00	uM	-25.590	
		31.25	uM	14.150	
		9.77	uM	50.690	
		3.05	uM	57.880	
		953.67	nM	38.900	
		298.02	nM	28.530	
		93.13	nM	19.660	
		29.10	nM	17.490	
		9.09	nM	-0.600	
		2.84	nM	-4.190	
		888.18	pM	4.670	
 59-0026	248.29				
59-0026		100.00	uM	-29.830	
		31.25	uM	-9.440	
		9.77	uM	-10.470	
		3.05	uM	46.220	
		953.67	nM	107.760	
		298.02	nM	86.720	
		93.13	nM	36.850	
		29.10	nM	26.720	
		9.09	nM	8.520	
		2.84	nM	-1.240	
		888.18	pM	4.020	

					
59-0027	250.30				
59-0027		100.00	uM	89.810	
		31.25	uM	54.670	
		9.77	uM	44.940	
		3.05	uM	23.780	
		953.67	nM	8.380	
		298.02	nM	6.330	
		93.13	nM	7.360	
		29.10	nM	3.380	
		9.09	nM	-1.620	
		2.84	nM	-3.670	
		888.18	pM	-0.720	
					
59-0028	226.28				
59-0028		100.00	uM	-26.750	
		31.25	uM	-16.740	
		9.77	uM	29.550	
		3.05	uM	100.580	
		953.67	nM	54.940	
		298.02	nM	31.340	
		93.13	nM	7.500	
		29.10	nM	7.500	
		9.09	nM	7.880	
		2.84	nM	3.140	
		888.18	pM	4.670	

			
59-0029	249.27		
59-0029		100.00 uM	-15.16
		31.25 uM	41.94
		9.77 uM	36.63
		3.05 uM	7.12
		953.67 nM	21.88
		298.02 nM	15.54
		93.13 nM	1.81
		29.10 nM	1.37
		9.09 nM	12.14
		2.84 nM	-4.23
		888.18 pM	9.04
			
59-0030	233.28		
59-0030		100.00 uM	-27.97
		31.25 uM	-22.83
		9.77 uM	-5.42
		3.05 uM	57.28
		953.67 nM	72.62
		298.02 nM	53.00
		93.13 nM	29.99
		29.10 nM	14.63
		9.09 nM	3.87
		2.84 nM	6.97
		888.18 pM	1.81
			
59-0031	231.30		
59-0031		100.00 uM	-25.79
		31.25 uM	-17.81
		9.77 uM	20.84
		3.05 uM	87.38
		953.67 nM	49.32
		298.02 nM	43.11
		93.13 nM	29.53
		29.10 nM	1.81
		9.09 nM	1.22
		2.84 nM	-0.55
		888.18 pM	4.16

			
59-0032	248.29		
59-0032		100.00 uM	-7.78
		31.25 uM	40.75
		9.77 uM	42.82
		3.05 uM	25.70
		953.67 nM	31.17
		298.02 nM	34.41
		93.13 nM	3.57
		29.10 nM	4.32
		9.09 nM	-10.00
		2.84 nM	5.65
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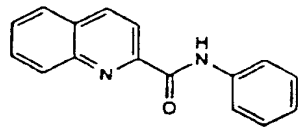			
59-0033	248.29		
59-0033		100.00 uM	-28.18
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		9.77 uM	55.30
		3.05 uM	49.71
		953.67 nM	47.41
		298.02 nM	0.25
		93.13 nM	7.98
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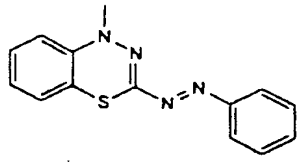
		9.09 nM	-7.63
		2.84 nM	-0.40
		888.18 pM	-5.98
			
59-0034	268.34		
59-0034		100.00 uM	-28.51
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		9.77 uM	73.58
		3.05 uM	37.91
		953.67 nM	20.09
		298.02 nM	16.87
		93.13 nM	15.23
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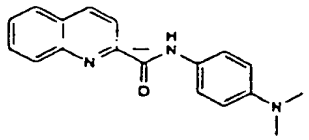
		9.09 nM	9.08
		2.84 nM	23.02
		888.18 pM	-0.32

			
59-0035	291.36		
59-0035		100.00uM	-14.92
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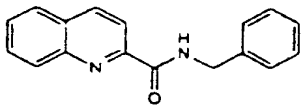
		9.77uM	15.87
		3.05uM	18.8
		953.67nM	3.88
		298.02nM	6.15
		93.13nM	3.22
		29.10nM	-10.03
		9.09nM	15.58
		2.84nM	-3.56
		888.18pM	-7.13
			
59-0036	262.31		
59-0036		100.00uM	-0.98
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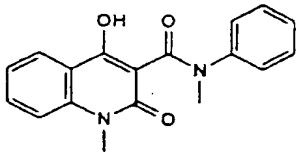
		9.77uM	-4.54
		3.05uM	-1.95
		953.67nM	0.32
		298.02nM	-6.49
		93.13nM	-17.19
		29.10nM	-0.66
		9.09nM	-5.52
		2.84nM	-9.4
		888.18pM	-16.53
			
59-0037	308.00		
59-0037		100.00uM	-10.69
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		9.77uM	-10.03
		3.05uM	-19.11
		953.67nM	-9.4
		298.02nM	2.27
		93.13nM	-2.9
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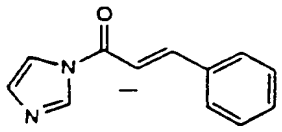
		9.09nM	2.59
		2.84nM	0.66
		888.18pM	-2.59

			
59-0038	291.36		
59-0038		100.00 uM	-23.43
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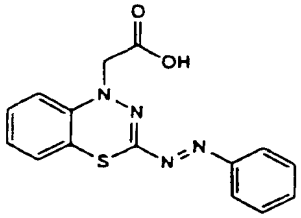
		9.77 uM	-0.10
		3.05 uM	-2.86
		953.67 nM	-2.24
		298.02 nM	3.90
		93.13 nM	6.35
		29.10 nM	1.15
		9.09 nM	6.96
		2.84 nM	-4.39
		888.18 pM	-0.38
			
59-0039	312.35		
59-0039		100.00 uM	14.17
		31.25 uM	7.62
		9.77 uM	1.94
		3.05 uM	-3.14
		953.67 nM	-7.77
		298.02 nM	-5.98
		93.13 nM	-8.82
		29.10 nM	-2.39
		9.09 nM	-16.58
		2.84 nM	-4.48
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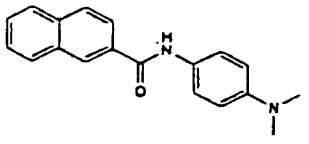
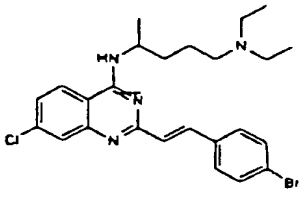
			
59-0040	290.37		
59-0040		100.00 uM	-20.40
		31.25 uM	-17.31
		9.77 uM	-8.11
		3.05 uM	32.18
		953.67 nM	36.18
		298.02 nM	17.44
		93.13 nM	2.04
		29.10 nM	10.35
		9.09 nM	-6.07
		2.84 nM	6.96
		888.18 pM	13.44

			
59-0041	501.90		
59-0041		100.00 uM	-18.37
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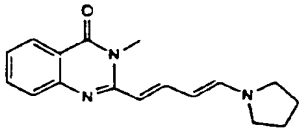
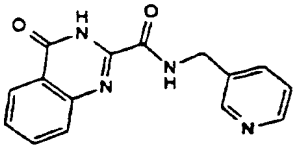
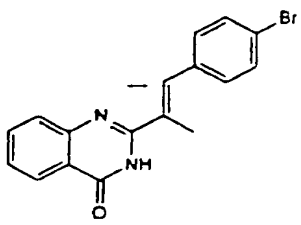
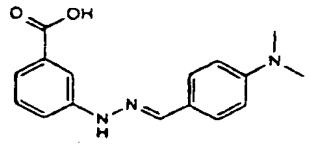
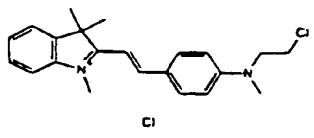
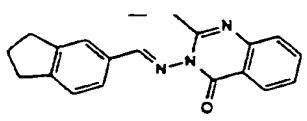
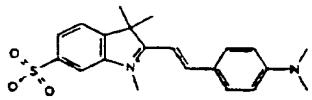
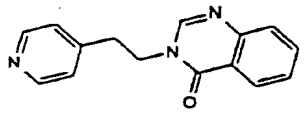
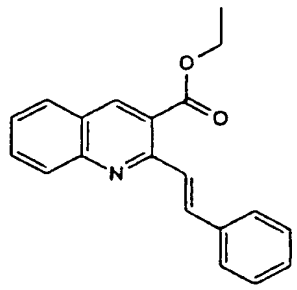
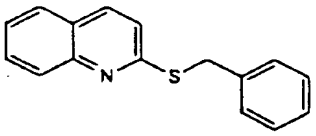
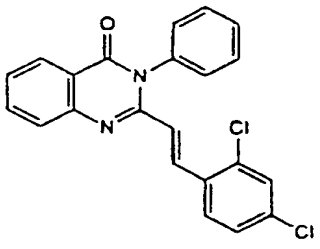
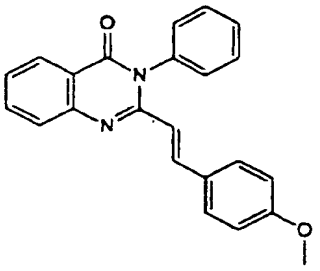
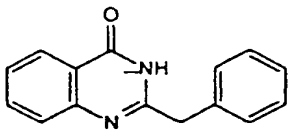
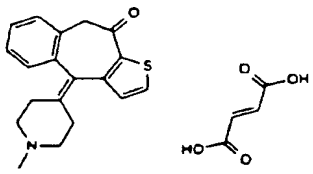
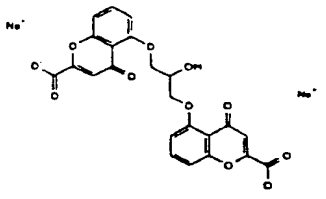
		9.77 uM	-5.11
		3.05 uM	3.31
		953.67 nM	-0.77
		298.02 nM	-1.56
		93.13 nM	3.55
		29.10 nM	-11.24
		9.09 nM	0.25
		2.84 nM	-0.27
		888.18 pM	2.02
			
59-0042	281.36		
59-0042		100.00 uM	163.51
		31.25 uM	-7.67
		9.77 uM	9.41
		3.05 uM	0.75
		953.67 nM	6.11
		298.02 nM	3.82
		93.13 nM	2.54
		29.10 nM	4.07
		9.09 nM	-9.73
		2.84 nM	-0.02
		888.18 pM	18.37
			
59-0043	280.29		
59-0043		100.00 uM	20.66
		31.25 uM	7.41
		9.77 uM	-1.29
		3.05 uM	-2.31
		953.67 nM	1.54
		298.02 nM	-0.79
		93.13 nM	1.52
		29.10 nM	2.79
		9.09 nM	-0.27
		2.84 nM	8.92
		888.18 pM	-4.34

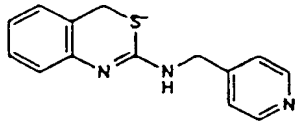
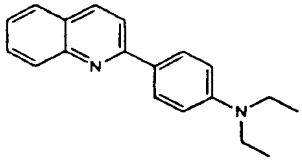
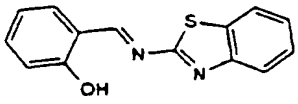
				
59-0044	341.21			
59-0044		100.00uM	7.38	
		31.25uM	11.72	
		9.77uM	12.49	
		3.05uM	-0.52	
		953.67nM	0.5	
		298.02nM	6.11	
		93.13nM	-1.54	
		29.10nM	19.14	
		9.09nM	7.13	
		2.84nM	-2.06	
		888.18pM	5.84	
				
59-0045	283.33			
59-0045		100.00uM	52.37	64.460
		31.25uM	148.43	192.960
		9.77uM	204.47	422.540
		3.05uM	280.3	437.020
		953.67nM	254.82	410.890
		298.02nM	218.21	266.090
		93.13nM	196.98	183.730
		29.10nM	96.06	80.440
		9.09nM	67.35	55.530
		2.84nM	52.99	44.160
				
59-0046	389.37			
59-0046		100.00uM	79.33	
		31.25uM	2.24	
		9.77uM	-1.67	
		3.05uM	-6.18	
		953.67nM	0.001	
		298.02nM	-3.83	
		93.13nM	-0.84	
		29.10nM	-8.42	
		9.09nM	3.92	
		2.84nM	0.3	
		888.18pM	5.61	

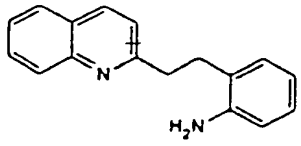
					
59-0047	303.37				
59-0047		100.00	uM	-6.73	
		31.25	uM	10.38	
		9.77	uM	-6.16	
		3.05	uM	-1.39	
		953.67	nM	-10.11	
		298.02	nM	-4.49	
		93.13	nM	-7.28	
		29.10	nM	-12.34	
		9.09	nM	-3.08	
		2.84	nM	-2.28	
		888.18	pM	-5.34	
					
59-0048	384.50				
59-0048		100.00	uM	-6.73	
		31.25	uM	0.27	
		9.77	uM	-5.61	
		3.05	uM	-2.28	
		953.67	nM	-12.89	
		298.02	nM	-1.69	
		93.13	nM	-4.77	
		29.10	nM	-8.14	
		9.09	nM	-3.92	
		2.84	nM	-11.2	
		888.18	pM	-4.77	
					
59-0049	251.29				
59-0049		100.00	uM	4.49	
		31.25	uM	0	
		9.77	uM	-4.77	
		3.05	uM	1.96	
		953.67	nM	8.69	
		298.02	nM	-5.04	
		93.13	nM	-2.24	
		29.10	nM	1.69	
		9.09	nM	-4.49	
		2.84	nM	2.24	
		888.18	pM	-0.3	

					
59-0050	303.36				
59-0050		100.00	uM	45.79	
		31.25	uM	10.02	
		9.77	uM	11.29	
		3.05	uM	-4.68	
		953.67	nM	-6.92	
		298.02	nM	-5.65	
		93.13	nM	1.69	
		29.10	nM	-7.57	
		9.09	nM	-12.05	
		2.84	nM	-13.63	
		888.18	pM	5.2	
					
59-0051	251.35				
59-0051		100.00	uM	32.36	
		31.25	uM	-18.42	
		9.77	uM	-0.55	
		3.05	uM	-13.94	
		953.67	nM	-12.02	
		298.02	nM	-14.59	
		93.13	nM	-7.55	
		29.10	nM	-11.4	
		9.09	nM	-14.91	
		2.84	nM	-10.74	
		888.18	pM	-20.03	

					
59-0052	393.28				
59-0052		100.00	uM	-21.62	
		31.25	uM	-13.32	
		9.77	uM	-21.31	
		3.05	uM	-11.08	
		953.67	nM	-20.66	
		298.02	nM	-17.14	
		93.13	nM	-16.49	
		29.10	nM	-11.4	
		9.09	nM	-10.74	
		2.84	nM	-11.08	
		888.18	pM	-14.59	
					
59-0053	354.41				
59-0053		100.00	uM	-17.14	
		31.25	uM	-21.31	
		9.77	uM	-9.47	
		3.05	uM	-11.08	
		953.67	nM	-0.83	
		298.02	nM	-11.4	
		93.13	nM	-9.47	
		29.10	nM	-19.72	
		9.09	nM	-18.45	
		2.84	nM	-10.09	
		888.18	pM	-2.76	

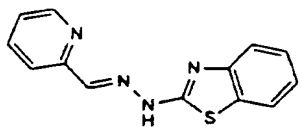
			
59-0054	236.28		
59-0054		100.00 uM	-20.04
		31.25 uM	-6.95
		9.77 uM	8.3
		3.05 uM	-3.37
		953.67 nM	-2.4
		298.02 nM	-0.99
		93.13 nM	-0.99
		29.10 nM	-1.94
		9.09 nM	5.92
		2.84 nM	-2.17
		888.18 pM	-9.31
			
59-0055	425.51		
59-0055		100.00 uM	-13.76
		31.25 uM	-9.51
		9.77 uM	-2.02
		3.05 uM	3.24
		953.67 nM	-6.27
		298.02 nM	-4.05
		93.13 nM	-1.62
		29.10 nM	-7.49
		9.09 nM	-7.09
		2.84 nM	-3.04
			
59-0056	512.34		
59-0056		100.00 uM	-1.42
		31.25 uM	-4.87
		9.77 uM	0.18
		3.05 uM	3.84
		953.67 nM	-5.07
		298.02 nM	-7.29
		93.13 nM	0.001
		29.10 nM	-4.25
		9.09 nM	-1.02
		2.84 nM	-3.85

		9.09 nM	8.070
		2.84 nM	0.440
 59-0063			
59-0063		100.00 uM	-2.510
		31.25 uM	-6.130
		9.77 uM	-8.950
		3.05 uM	-8.020
		953.67 nM	-8.010
		298.02 nM	-2.520
		93.13 nM	-5.810
		29.10 nM	-3.450
		9.09 nM	-4.390
		2.84 nM	-6.280
 59-0064			
59-0064		100.00 uM	-23.090
		31.25 uM	-21.040
		9.77 uM	78.400
		3.05 uM	155.220
		953.67 nM	113.120
		298.02 nM	30.640
		93.13 nM	15.240
		29.10 nM	22.150
		9.09 nM	-0.770
		2.84 nM	4.410
 59-0065			
59-0065		100.00 uM	-2.030
		31.25 uM	-2.980
		9.77 uM	-15.240
		3.05 uM	-15.400
		953.67 nM	-15.240
		298.02 nM	-10.520
		93.13 nM	-13.830
		29.10 nM	-5.810
		9.09 nM	-3.620
		2.84 nM	-7.070



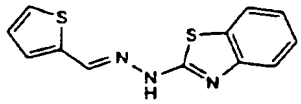
59-0066

59-0066	100.00 uM	10.060
	31.25 uM	2.680
	9.77 uM	10.850
	3.05 uM	14.610
	953.67 nM	0.950
	298.02 nM	3.780
	93.13 nM	1.730
	29.10 nM	-2.820
	9.09 nM	-2.820
	2.84 nM	-3.920



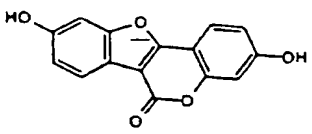
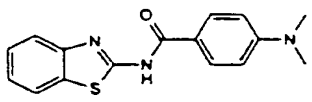
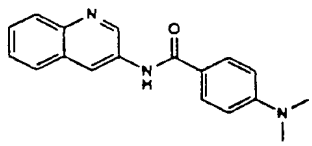
59-0067

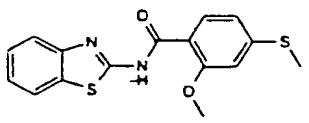
59-0067	100.00 uM	-24.040
	31.25 uM	-24.890
	9.77 uM	-1.450
	3.05 uM	60.900
	953.67 nM	133.860
	298.02 nM	75.330
	93.13 nM	28.760
	29.10 nM	20.070
	9.09 nM	4.980
	2.84 nM	4.450



59-0068

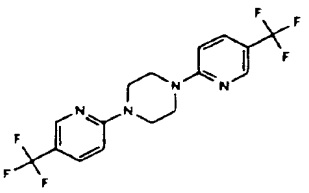
59-0068	100.00 uM	-22.130
	31.25 uM	-7.880
	9.77 uM	93.900
	3.05 uM	81.060
	953.67 nM	22.330
	298.02 nM	17.300
	93.13 nM	8.460
	29.10 nM	-3.530
	9.09 nM	-4.230
	2.84 nM	-6.140

			
59-0069			
59-0069		100.00 uM	5.490
		31.25 uM	9.670
		9.77 uM	16.090
		3.05 uM	-7.180
		953.67 nM	-2.840
		298.02 nM	-3.710
		93.13 nM	-11.180
		29.10 nM	-5.790
		9.09 nM	-7.180
		2.84 nM	-4.750
			
59-0070			
59-0070		100.00 uM	-25.930
		31.25 uM	-23.000
		9.77 uM	36.060
		3.05 uM	214.280
		953.67 nM	158.530
		298.02 nM	72.890
		93.13 nM	20.940
		29.10 nM	7.760
		9.09 nM	7.590
		2.84 nM	-8.400
			
59-0071			
59-0071		100.00 uM	-18.650
		31.25 uM	-15.540
		9.77 uM	17.060
		3.05 uM	176.090
		953.67 nM	76.070
		298.02 nM	31.260
		93.13 nM	16.410
		29.10 nM	4.870
		9.09 nM	-7.330
		2.84 nM	-4.660



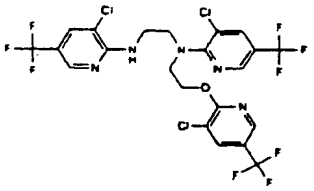
59-0072

59-0072	100.00 uM	-19.750
	31.25 uM	-18.650
	9.77 uM	-16.430
	3.05 uM	-15.770
	953.67 nM	9.970
	298.02 nM	74.740
	93.13 nM	175.430
	29.10 nM	213.580
	9.09 nM	164.320
	2.84 nM	119.100
	888.18 pM	60.770



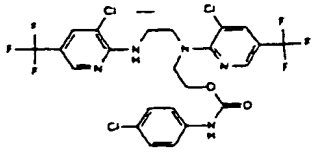
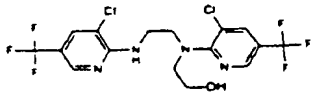
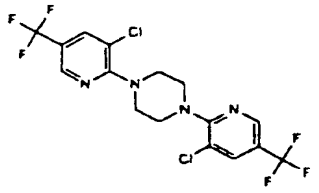
59-0073

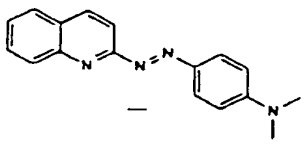
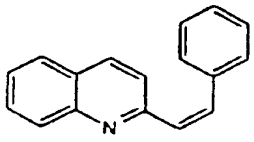
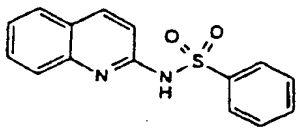
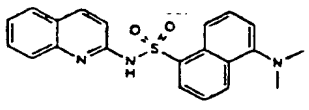
59-0073	100.00 uM	-3.010
	31.25 uM	-4.830
	9.77 uM	-9.660
	3.05 uM	-4.680
	953.67 nM	-6.500
	298.02 nM	-2.510
	93.13 nM	7.140
	29.10 nM	0.97
	9.09 nM	-5.5
	2.84 nM	5.3

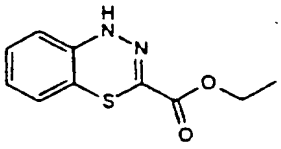
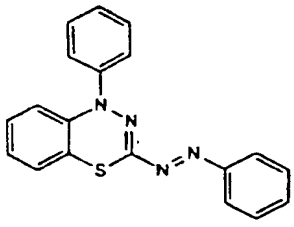
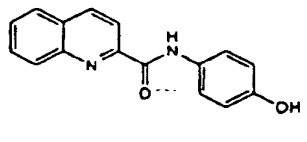


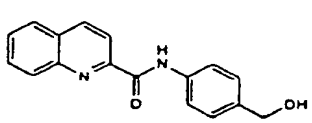
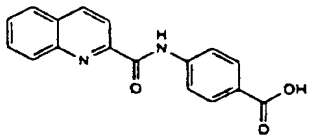
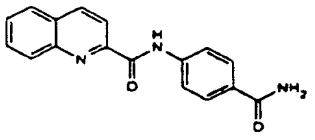
59-0074

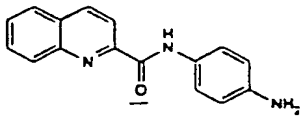
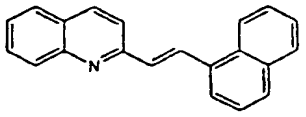
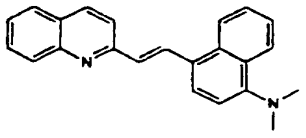
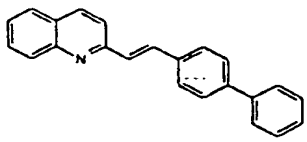
59-0074	100.00 uM	-2.85
	31.25 uM	2.14
	9.77 uM	-4.85
	3.05 uM	-3.5
	953.67 nM	-4.85
	298.02 nM	9.95
	93.13 nM	4.47
	29.10 nM	-8
	9.09 nM	-4.17
	2.84 nM	6.97

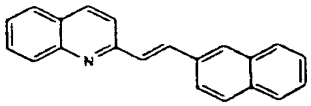
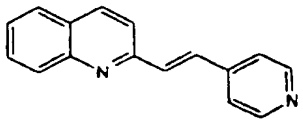
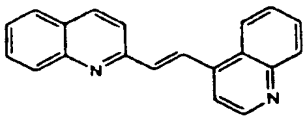
				
59-0075				
59-0075		100.00	uM	-0.68
		31.25	uM	-10.16
		9.77	uM	-5.35
		3.05	uM	-6.5
		953.67	nM	-0.85
		298.02	nM	5.97
		93.13	nM	0.97
		29.10	nM	-2.35
		9.09	nM	0.32
		2.84	nM	10.47
				
59-0076				
59-0076		100.00	uM	-19.12
		31.25	uM	9.29
		9.77	uM	10.63
		3.05	uM	22.43
		953.67	nM	19.93
		298.02	nM	3.47
		93.13	nM	19.93
		29.10	nM	10.63
		9.09	nM	14.28
		2.84	nM	11.3
				
59-0077				
59-0077		100.00	uM	-20.96
		31.25	uM	-16.23
		9.77	uM	-10.58
		3.05	uM	-11.96
		953.67	nM	-19.44
		298.02	nM	-17.3
		93.13	nM	-13.79
		29.10	nM	-15.62
		9.09	nM	-14.09
		2.84	nM	-14.4

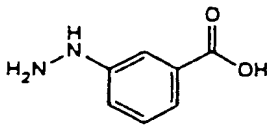
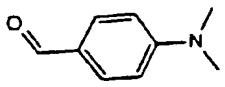
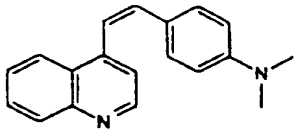
			
59-0078			
		100.00 uM	-26.54
		31.25 uM	-22.56
		9.77 uM	71.53
		3.05 uM	207.96
		953.67 nM	379.23
		298.02 nM	241.46
		93.13 nM	136.10
		29.10 nM	84.02
		9.09 nM	50.35
		2.84 nM	56.60
		0.80 nM	92.52
			
59-0079			
59-0079		100.00 uM	-34.98
		31.25 uM	-21.39
		9.77 uM	37.20
		3.05 uM	122.58
		953.67 nM	69.01
		298.02 nM	64.00
		93.13 nM	46.49
		29.10 nM	30.31
		9.09 nM	33.49
		2.84 nM	29.76
			
59-0080			
59-0080		100.00 uM	5.39
		31.25 uM	5.56
		9.77 uM	6.44
		3.05 uM	2.44
		953.67 nM	-5.03
		298.02 nM	7.66
		93.13 nM	-3.63
		29.10 nM	3.65
		9.09 nM	1.05
		2.84 nM	6.94
			—
59-00A			

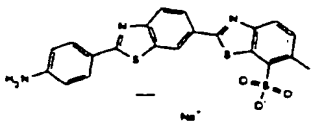
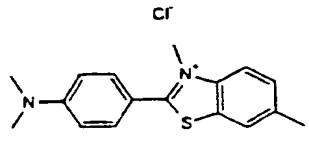
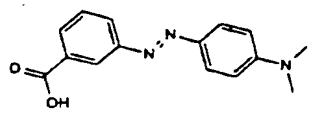
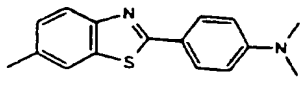
59-0081		100.00 uM	62.840
		31.25 uM	11.300
		9.77 uM	-8.670
		3.05 uM	2.440
		953.67 nM	-5.200
		298.02 nM	-2.080
		93.13 nM	1.220
		29.10 nM	-2.250
		9.09 nM	1.050
		2.84 nM	-3.300
 59-0082			
59-0082		100.00 uM	111.79
		31.25 uM	62.68
		9.77 uM	32.36
		3.05 uM	9.11
		953.67 nM	-10.62
		298.02 nM	-1.86
		93.13 nM	-6.89
		29.10 nM	-3.91
		9.09 nM	2.22
		2.84 nM	16.36
 59-0083			
59-0083		100.00 uM	48.93
		31.25 uM	40.91
		9.77 uM	25.85
		3.05 uM	17.85
		953.67 nM	8.55
		298.02 nM	3.9
		93.13 nM	2.05
		29.10 nM	7.99
		9.09 nM	-3.91
		2.84 nM	3.35
 59-0084			
59-0084		100.00 uM	37.670
		31.25 uM	26.050
		9.77 uM	9.210
		3.05 uM	10.070

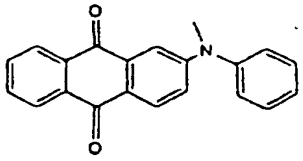
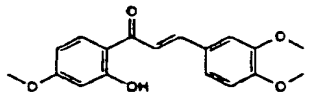
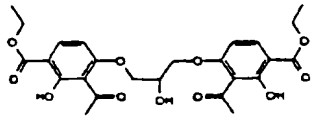
		953.67 nM	21.700
		298.02 nM	5.900
		93.13 nM	4.870
		29.10 nM	-10.920
		9.09 nM	10.080
		2.84 nM	-2.080
 59-0085			
59-0085		100.00 uM	17.070
		31.25 uM	41.890
		9.77 uM	18.500
		3.05 uM	20.340
		953.67 nM	22.490
		298.02 nM	8.090
		93.13 nM	11.780
		29.10 nM	1.240
		9.09 nM	-0.760
		2.84 nM	5.940
 59-0086			
59-0086		100.00 uM	30.750
		31.25 uM	31.190
		9.77 uM	14.790
		3.05 uM	13.500
		953.67 nM	14.080
		298.02 nM	3.940
		93.13 nM	9.370
		29.10 nM	-2.610
		9.09 nM	-5.040
		2.84 nM	1.530
 59-0087			
59-0087		100.00 uM	10.660
		31.25 uM	11.080
		9.77 uM	3.100
		3.05 uM	-1.320
		953.67 nM	17.070
		298.02 nM	7.950
		93.13 nM	-4.460
		29.10 nM	4.510
		9.09 nM	-0.470
		2.84 nM	9.660

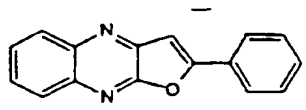
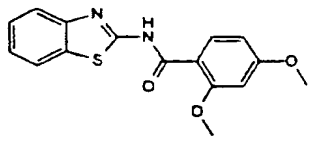
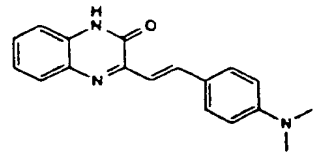
 59-0088			
59-0088	100.00 uM		
	31.25 uM		
	9.77 uM		
	3.05 uM		
	953.67 nM		
	298.02 nM		
	93.13 nM		
	29.10 nM		
	9.09 nM		
	2.84 nM		
 59-0089			
59-0089	100.00 uM	60.09	
	31.25 uM	116.25	
	9.77 uM	65.84	
	3.05 uM	36.1	
	953.67 nM	37.96	
	298.02 nM	18.42	
	93.13 nM	8.33	
	29.10 nM	13.58	
	9.09 nM	0.75	
	2.84 nM	-5.77	
 59-0090			
59-0090	100.00 uM	32.77	
	31.25 uM	24.63	
	9.77 uM	19.5	
	3.05 uM	41.31	
	953.67 nM	9.8	
	298.02 nM	-1.76	
	93.13 nM	3.53	
	29.10 nM	2.95	
	9.09 nM	2.95	
	2.84 nM	7.8	
 59-0091		—	
59-0091	100.00 uM	0.26	
	31.25 uM	13.54	

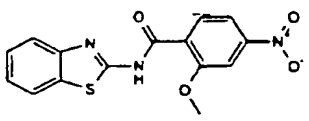
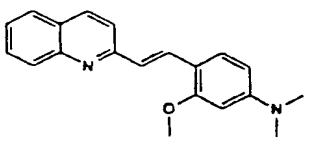
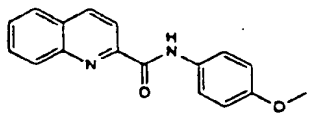
	9.77|uM	95.94|
	3.05|uM	87.71|
	953.67|nM	44.17|
	298.02|nM	38.26|
	93.13|nM	23.87|
	29.10|nM	21.65|
	9.09|nM	10.95|
	2.84|nM	20.92|
 59-0092		
59-0092	100.00|uM	-11.56|
	31.25|uM	17.84|
	9.77|uM	50.19|
	3.05|uM	25.84|
	953.67|nM	14.4|
	298.02|nM	6.77|
	93.13|nM	8.62|
	29.10|nM	2.22|
	9.09|nM	8.38|
	2.84|nM	1|
 59-0093		
59-0093	100.00|uM	-11.67|
	31.25|uM	15.02|
	9.77|uM	35.44|
	3.05|uM	29.89|
	953.67|nM	22.88|
	298.02|nM	19.56|
	93.13|nM	5.18|
	29.10|nM	7.39|
	9.09|nM	4.56|
	2.84|nM	5.9|
 59-0094		
59-0094	100.00|uM	-17.69|
	31.25|uM	45.15|
	9.77|uM	24.97|
	3.05|uM	19.81|
	953.67|nM	9.35|
	298.02|nM	1.36|
	93.13|nM	9.24|
	29.10|nM	-0.48|
	9.09|nM	6.16|
	2.84|nM	1.81|

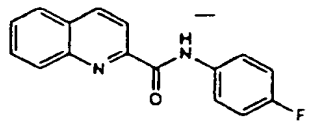
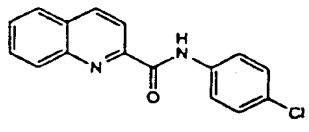
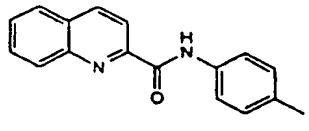
		93.13 nM	10.68
		29.10 nM	5.89
		9.09 nM	5.45
		2.84 nM	10.24
		0.80 nM	4.14
 59-0111	152.15		
		100.00 uM	23.360
		31.25 uM	22.330
		9.77 uM	12.260
		3.05 uM	5.390
		953.67 nM	2.190
		298.02 nM	1.230
		93.13 nM	2.430
		29.10 nM	6.350
		9.09 nM	4.350
		2.84 nM	4.350
		0.80 nM	3.230
 59-0112	149.19		
		100.00 uM	2.670
		31.25 uM	4.670
		9.77 uM	2.750
		3.05 uM	3.790
		953.67 nM	4.270
		298.02 nM	1.150
		93.13 nM	9.630
		29.10 nM	0.920
		9.09 nM	0.510
		2.84 nM	12.900
		0.80 nM	2.990
 59-0113	274.37		
		100.00 uM	22.010
		31.25 uM	25.940
		9.77 uM	7.500
		3.05 uM	3.070
		953.67 nM	-0.760
		298.02 nM	-4.690
		93.13 nM	-4.790
		29.10 nM	5.090
		9.09 nM	0.150
		2.84 nM	-0.250
		0.80 nM	0.150

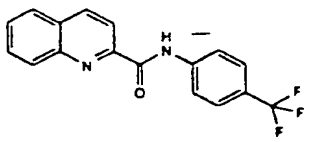
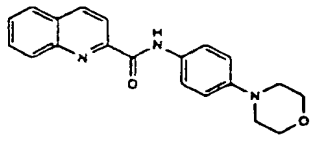
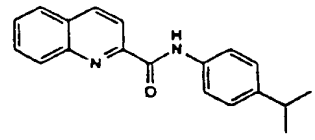
					
59-0114	475.54				
		100.00	uM	52.030	
		31.25	uM	36.120	
		9.77	uM	25.840	
		3.05	uM	16.670	
		953.67	nM	12.540	
		298.02	nM	9.420	
		93.13	nM	-1.060	
		29.10	nM	2.160	
		9.09	nM	-6.000	
		2.84	nM	2.470	
		0.80	nM	-1.460	
					
59-0115	318.87				
		100.00	uM	73.700	
		31.25	uM	2.770	
		9.77	uM	-10.430	
		3.05	uM	-12.340	
		953.67	nM	-13.750	
		298.02	nM	-13.960	
		93.13	nM	-11.940	
		29.10	nM	-9.830	
		9.09	nM	-6.820	
		2.84	nM	-0.950	
		0.80	nM	-0.050	
					
59-0116	269.30				
		100.00	uM	31.380	
		31.25	uM	109.060	
		9.77	uM	231.070	
		3.05	uM	240.670	
		953.67	nM	132.020	
		298.02	nM	75.820	
		93.13	nM	53.250	
		29.10	nM	47.500	
		9.09	nM	39.440	
		2.84	nM	42.170	
		0.80	nM	31.180	
					
59-0117	268.38				
		100.00	uM	-68.520	

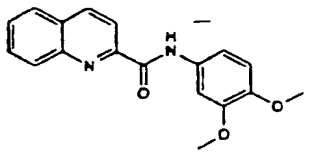
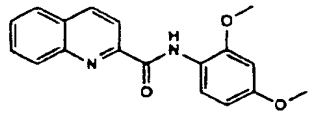
		31.25 uM	-7.450
		9.77 uM	111.630
		3.05 uM	84.340
		953.67 nM	4.740
		298.02 nM	-19.270
		93.13 nM	-26.660
		29.10 nM	-28.880
		9.09 nM	-42.180
		2.84 nM	-41.300
		0.80 nM	-39.220
			
59-0118	313.36		
		100.00 uM	-67.170
		31.25 uM	-56.580
		9.77 uM	-58.060
		3.05 uM	-55.720
		953.67 nM	-48.200
		298.02 nM	-50.300
		93.13 nM	-33.310
		29.10 nM	-47.340
		9.09 nM	-49.310
		2.84 nM	-56.200
		0.80 nM	-57.310
			
59-0119	314.34		
		100.00 uM	167.500
		31.25 uM	-29.240
		9.77 uM	-57.800
		3.05 uM	-52.030
		953.67 nM	-54.240
		298.02 nM	-53.870
		93.13 nM	-38.110
		29.10 nM	-55.100
		9.09 nM	-52.270
		2.84 nM	-53.500
		0.80 nM	-43.650
			
59-0120	504.49		
		100.00 uM	-82.790
		31.25 uM	-80.470
		9.77 uM	-68.800
		3.05 uM	-60.790
		953.67 nM	-54.240
		298.02 nM	-45.250
		93.13 nM	-50.660

		2.84 nM	6.27
		0.80 nM	3.55
 59-0146	248.27		
		100.00 uM	-83.05
		31.25 uM	4.42
		9.77 uM	-13.73
		3.05 uM	-16.45
		953.67 nM	-35.47
		298.02 nM	-51.25
		93.13 nM	-50.13
		29.10 nM	-42.92
		9.09 nM	-45.64
		2.84 nM	-56.58
		0.80 nM	-39.68
 59-0147	314.36		
		100.00 uM	-85
		31.25 uM	-85
		9.77 uM	-80.29
		3.05 uM	-41.67
		953.67 nM	78.69
		298.02 nM	269.13
		93.13 nM	323.59
		29.10 nM	339.88
		9.09 nM	270.48
		2.84 nM	245.58
		0.80 nM	180.33
 59-0148	291.35		
		100.00 uM	-68.38
		31.25 uM	-36.33
		9.77 uM	-2.3
		3.05 uM	12.12
		953.67 nM	-2.42
		298.02 nM	-16.21
		93.13 nM	-30.87
		29.10 nM	-35.58
		9.09 nM	-39.07
		2.84 nM	-41.18
		0.80 nM	-45.53

			
59-0149	329.33		
		100.00 uM	-16.9
		31.25 uM	-1.8
		9.77 uM	-0.53
		3.05 uM	15.29
		953.67 nM	78.78
		298.02 nM	163.5
		93.13 nM	223.57
		29.10 nM	173.93
		9.09 nM	122.3
		2.84 nM	98.02
		0.80 nM	69.06
			
59-0150	304.39		
		100.00 uM	63.32
		31.25 uM	193.53
		9.77 uM	419.26
		3.05 uM	497.21
		953.67 nM	295.19
		298.02 nM	193.35
		93.13 nM	99.48
		29.10 nM	69.96
		9.09 nM	59
		2.84 nM	52.16
		0.80 nM	48.75
			
59-0151	278.311		
59-0151		100.00 uM	-6.660
		31.25 uM	16.240
		9.77 uM	18.300
		3.05 uM	11.690
		953.67 nM	8.500
		298.02 nM	9.070
		93.13 nM	6.110
		29.10 nM	5.880
		9.09 nM	7.700
		2.84 nM	2.000
		0.80 nM	1.210

						
59-0152	266.275					
59-0152		100.00	uM	-6.890		
		31.25	uM	12.490		
		9.77	uM	21.950		
		3.05	uM	12.820		
		953.67	nM	7.350		
		298.02	nM	4.290		
		93.13	nM	9.750		
		29.10	nM	4.860		
		9.09	nM	1.320		
		2.84	nM	4.280		
		0.80	nM	4.160		
						
59-0153	282.73					
59-0153		100.00	uM	-4.150		
		31.25	uM	-0.390		
		9.77	uM	11.120		
		3.05	uM	14.540		
		953.67	nM	9.520		
		298.02	nM	11.570		
		93.13	nM	-0.160		
		29.10	nM	1.550		
		9.09	nM	-0.960		
		2.84	nM	4.730		
		0.80	nM	5.650		
						
59-0154	262.312					
59-0154		100.00	uM	0.290		
		31.25	uM	24.670		
		9.77	uM	15.680		
		3.05	uM	14.540		
		953.67	nM	13.170		
		298.02	nM	5.540		
		93.13	nM	2.690		
		29.10	nM	-1.190		
		9.09	nM	2.460		
		2.84	nM	4.170		
		0.80	nM	1.890		

						
59-0155	316.282					
59-0155		100.00	uM	-2.950		
		31.25	uM	1.900		
		9.77	uM	-9.450		
		3.05	uM	-0.220		
		953.67	nM	0.690		
		298.02	nM	5.090		
		93.13	nM	-3.250		
		29.10	nM	0.530		
		9.09	nM	-1.900		
		2.84	nM	9.480		
		0.80	nM	-1.130		
						
59-0156	333.391					
59-0156		100.00	uM	5.840		
		31.25	uM	2.050		
		9.77	uM	7.960		
		3.05	uM	6.890		
		953.67	nM	-0.370		
		298.02	nM	-1.880		
		93.13	nM	-3.550		
		29.10	nM	-7.340		
		9.09	nM	-1.590		
		2.84	nM	2.650		
		0.80	nM	2.500		
						
59-0157	290.366					
59-0157		100.00	uM	-6.440		
		31.25	uM	14.920		
		9.77	uM	19.930		
		3.05	uM	11.440		
		953.67	nM	8.570		
		298.02	nM	-7.190		
		93.13	nM	0.080		
		29.10	nM	-0.230		
		9.09	nM	-4.460		
		2.84	nM	2.200		
		0.80	nM	9.920		

						
59-0158	308.337					
59-0158		100.00	uM	-5.980		
		31.25	uM	3.720		
		9.77	uM	16.140		
		3.05	uM	27.060		
		953.67	nM	9.930		
		298.02	nM	11.900		
		93.13	nM	2.810		
		29.10	nM	3.110		
		9.09	nM	0.690		
		2.84	nM	1.900		
		0.80	nM	7.970		
						
59-0159	308.337					
59-0159		100.00	uM	2.790		
		31.25	uM	13.530		
		9.77	uM	4.700		
		3.05	uM	10.910		
		953.67	nM	2.800		
		298.02	nM	9.710		
		93.13	nM	4.830		
		29.10	nM	0.650		
		9.09	nM	5.900		
		2.84	nM	6.610		
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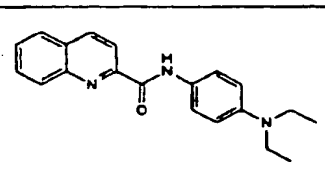						
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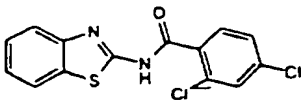						
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		9.09	nM			
		2.84	nM			
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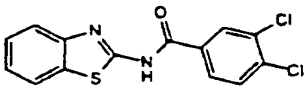						
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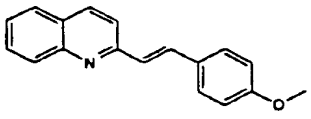						
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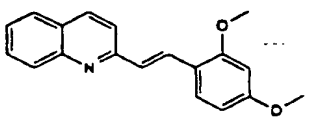						
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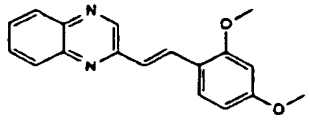 59-0203	292.338					
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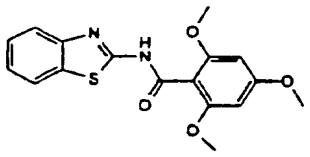 59-0204	344.389					
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		2.84	nM			
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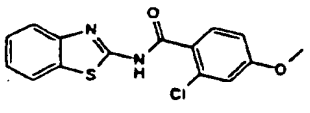 59-0205	318.782					
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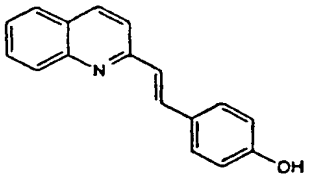
		9.09	nM			
		2.84	nM			
		0.80	nM			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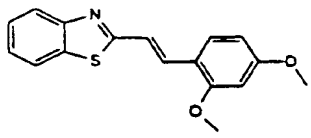						
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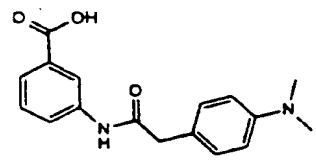						
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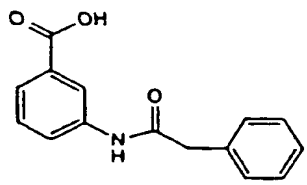						
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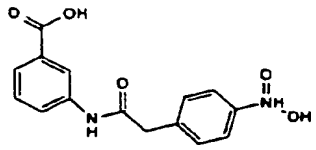						
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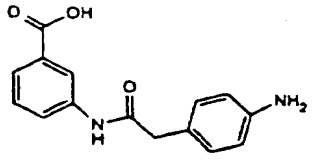						
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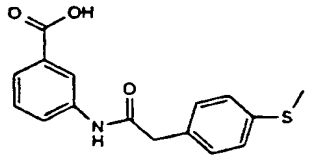						
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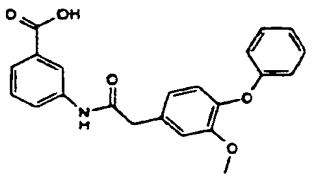						
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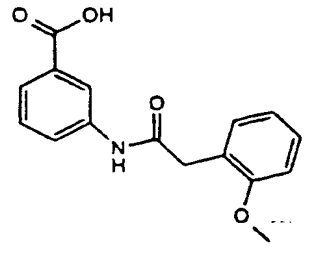						
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17019

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/54
US CL :514/222.8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/222.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS AND CAS ONLINE: compounds of the claims with bone, osteoporosis, hyperparathyroidism, periodontal, prosthetic, dental

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,280,040 A (LABROO ET AL.) 18 January 1994.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 FEBRUARY 1997	Date of mailing of the international search report 20 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KIMBERLY JORDAN Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*